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Contents

REVIEW ARTICLE

EXPORT POTENTIAL AND PACKAGING OF SOME IMPORTANT FRUITS OF INDIA

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Abstract: Fruits and vegetables are an important sub-sector in the agricultural sector because they are valued as protective food. They are very rich source of minerals, vitamins providing more energy per unit weight than cereals. India's is a country with wide agro-climatic conditions as a result of which we have got different climatic condition in different parts of country throughout the year. Because of this reason the production of fruits and vegetables is available in the country throughout the year in one or another part.

Keywords: Agriculture, Fruit, Production, Vegetables

INTRODUCTION

As a result, India ranks second in fruits and vegetable production in the world after china. As per NHB database during 2014-2015, India produced 86.602 million metric tonnes of fruits, 169.478 million metric tonnes of vegetables (Gandhi, 2015). The area under cultivation of fruits in 2014-15 was 6.110 million hectares whereas the area under vegetables was 9.542 million hectares (Gandhi, 2015). India ranks first in the production of Banana (22.94%), Papaya (44.03%) and Mangoes (37.57%). India rank first in production of many vegetables such as Ginger and Okra and ranks second in the production of Potato, Onion, Cauliflower, Brinjal and Cabbage.

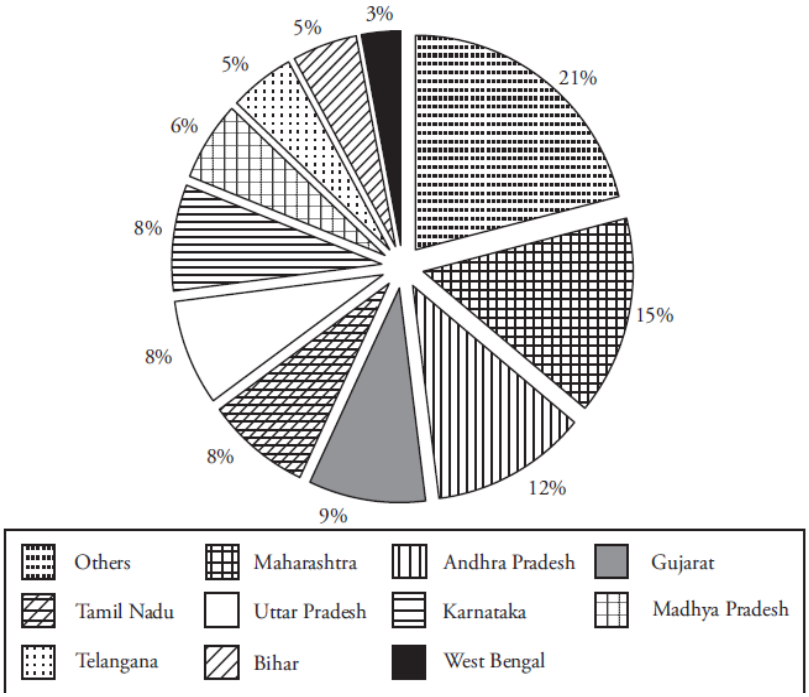

Fig. 1. Leading fruits producing states of 2013-14

During 2013-14, the total fruit production was highest in the case of Maharashtra (134.6 lakh tonnes) followed by Andhra Pradesh (105.11 lakh tonnes). The annual growth in citrus fruits is quite high (10.48%) during 2013-14. This fruit has been contributing 12–13% of total fruit production over the last few years. The graphical representation of production share of leading vegetable-producing states of 2013-14 is shown in Figure 1.
Source: Based on the data of Table 7.2.8. in Horticulture Statistics Division, DAC&FW

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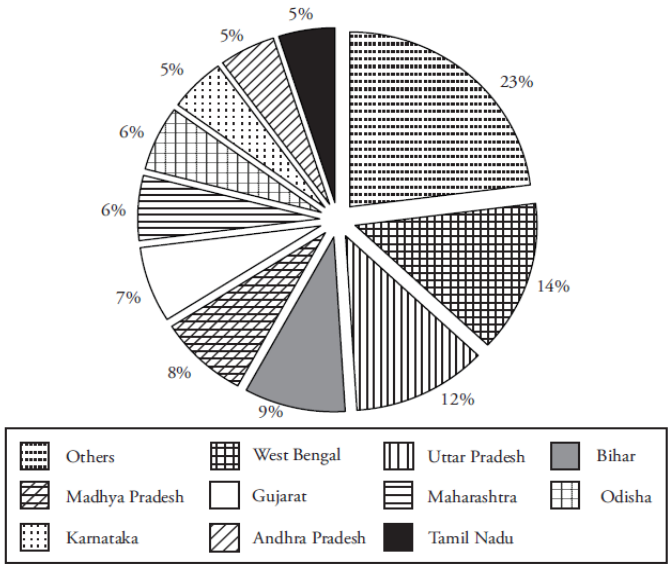

Fig. 2. Leading vegetables producing states of 2013-14

During 2013-14, the area under vegetables is estimated at 9.4 million hectares with a production of 162.9 million tonnes in India. For this period the total vegetable production was highest in case of West Bengal (23,045 thousand tonnes) followed by Uttar Pradesh (18,545 thousand tonnes). The graphical representation of production share of leading vegetable-producing states of 2013-14 is shown in Figure 2.

Source: Based on the data of Table 7.2.10. Horticulture Statistics Division, DAC&FW

The high level of production offer opportunities for tremendous export and can earn foreign exchequer and raise the economy of country and helps in increases GDP. India in 2015-16 exported fruits and vegetables worth Rs. 8,391.41 crores which constituted of fruits worth Rs. 3,524.50 and vegetables worth Rs. 4,866.91 crores (APEDA, 2016). India is largest exporter of Mangoes, Walnuts, Grapes, Banana and Pomegranate. Among the vegetables Onions, Okra, Bitter Gourd, Green Chilles, Mushrooms and Potatoes constitute major export basket of the vegetables. Now the major destination for fruits and vegetables are neighbouring countries UAE, Bangladesh, Malasiya, Srilanka. India export share in the global market is just 1%. There is an increasing acceptance of Indian fruits in many parts of world. However, Indian exporter and government chain is not strong enough to boost the Indian farmer and exporter to send this fruits and vegetables to different parts of the world. The present plans in last few years in private sector, public sector and APEDA assistance has helped to set up the chain for export of fruits and vegetables is a tough process as these are highly perishable. So capacity building initiatives are required at the level of farmers, exporters, processers in order to boost the export of fruits and vegetables to the developing countries in order to earn good foreign chequer.

Low availability of quality fruits and vegetables is mainly due to considerably high post-harvest losses, poor transportation, improper storage and low processing capacity with a growing population. The increased production of fruits and vegetables and other agricultural produce will be fully realised only when they reach the consumer in good condition and at a reasonable price. The post-harvest losses could be considerably reduced by adopting improved packaging, handling and efficient system of transport.

Packaging is an important consideration in vegetable and fruit market. The use of properly designed containers for transporting and marketing of vegetables can significantly reduce the losses and maintain their freshness succulence and quality for longer period (Stokes, 1974). The package must be capable of protecting the product from the transport hazards; preventing the microbial and insect damage; minimising the physiological and biochemical changes and losses in weight. Packaging is required not only for preservation and protection but also for safe transportation of products during storage and handling (APEDA, 2005). Increasing exports and stringent export market needs have also influenced the packaging trend. Packaging of fruits and vegetables is undertaken primarily to assemble the produce in convenient units for marketing and distribution.

Universal Product Codes (UPC or bar codes) may be included as part of the labelling (Erdei, 1993). The UPCs used in the food industry consist of a ten-digit machine readable code. The first five digits are a number assigned to the specific producer (packer or

shipper) and the second five digits represent specific product information such as type of produce and size of package. Although no price information is included, UPCs are used more and more by packers, shippers, buyers, and Example of a UPC retailers as a fast and convenient method of inventory control and cost accounting.

The package must stand up to long distance transportation, multiple handling, and the climate changes of different storage places, transport methods and market conditions. In designing fruit packages one should consider both the physiological characteristics of the fruit as well as the whole distribution network. Careful packing of fruits and vegetables is necessary to keep the produce in place with minimum shaking. Fruits and vegetables are normally packed in layers in crates and in each layer products are packed alternately placing the beak of one in between the shoulders of two. This method of packing is easy to follow and quick. It also provides enough room without compressing it.

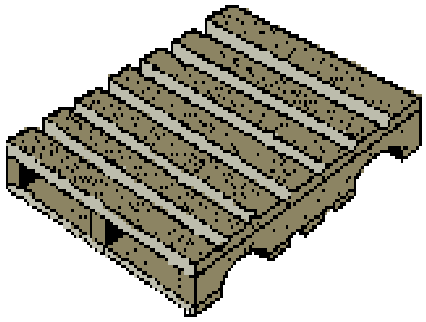

Fig. 3. Figure of Standard Size Pallets

Pallets literally form the base on which most fresh produce is delivered to the consumer (Fig. 3). Standard size pallets make efficient use of truck and van space and can accommodate heavier loads and more stress than lighter single-use pallets (National Wooden Pallet and Container Association. Washington, 1993)

Table 1. Some of the common cardboard boxes with Palletisation details:

S. No.	External Dimensions of the box LXBXH (MM)	Type of Pallet	No. of Boxes Per Layer (On Base)	Arrangement of Boxes on Pallet Base (LXW)	Base Utilization
1	400X200X150	IATA-A	75	15(W)X5(L)	91.76
		IATA-B	77	7(L)X11(W)	85.75
		EURO-A	12	3(L)X4(W)	100.00
		EURO-B	15	3(L)X5(W)	100.00
2	500X300X200	IATA-A	42	6(L)X7(W)	96.34
		IATA-B	42	6(L)X7(W)	87.70
		EURO-A	4	2(L)X2(W)	62.50
		EURO-B	8	4(W)X2(L)	100.00
3	600X300X200	IATA-A	35	5(L)X7(W)	96.34
		IATA-B	35	5(L)X7(W)	87.70
		EURO-A	4	2(L)X2(W)	75.00
		EURO-B	6	2(L)X3(W)	90.00
4	400X200X105	IATA-A	75	15(W)X5(L)	91.76
		IATA-B	77	7(L)X11(W)	85.75
		EURO-A	12	3(L)X4(W)	100.00
		EURO-B	15	3(L)X5(W)	100.00
5	350X220X100	IATA – A	78	13(L)X8(W)	91..55
		IATA – B	80	8(L)X10(W)	85.75
		EURO –A	10	5 (L)X2(W)	80.21
		EURO-B	12	3(L)X4(W)	77.00
6	260X230X100	IATA - A	104	13(L)X8(W)	95.11
		IATA - B	117	13(L)X9(W)	97.39
		EURO -A	12	4 (L)X3(W)	74.75
		EURO-B	16	4(L)X4(W)	79.73
7	225X170X100	IATA - A	117	13 (L)X4(W)	90.58
		IATA - B	130	13(L)X10(W)	91.61
		EURO -A	15	5 (L)X3(W)	79.10
		EURO-B	20	5(L)X4(W)	84.38

8	225X225X100	IATA - A	117	13(L)X9(W)	90.58
		IATA - B	130	13(L)X10(W)	91.61
		EURO -A	15	4 (L)X3(W)	74.75
		EURO-B	20	5(L)X4(W)	84.38
9	260X230X100	IATA - A	104	13(L)X8(W)	95.11
		IATA - B	117	13(L)X9(W)	97.39
		EURO -A	12	4 (L)X3(W)	74.75
		EURO-B	16	4(L)X4(W)	79.73
10	600X300X200	IATA - A	35	5(L)X7(W)	96.34
		IATA - B	35	5(L)X7(W)	87.70
		EURO -A	4	2 (L)X2(W)	75.00
		EURO-B	6	2(L)X3(W)	90.00

Source: APEDA, 2005

Specification details of Pressure Sensitive Tape & Reinforcement Strap

A. Pressure Sensitive Tap

i) **Material of Construction** : BOPP OR PVC
(biaxally oriented
polypropylene or
polyvinyl chloride)

ii) **Thickness** : 20 µ

iii)**Width (minimum)** : 50 mm

iv) **Adhesive Property** : as per is:3676-1986

for further specification details refer is: 2880-1978

b. Reinforcement Strap

i) **Material of construction** : pp (polypropylene)

ii) **Width (minimum)** : 12 mm

iii) **Thickness (minimum)** : 0.05 mm

iv) **Breaking load(min.)** : 80 kg/12 mm width

v) **Elongation (max.)** : 25%

Source: APEDA, 2005

Export potential and packaging for some of the important fruits with largest exported from India:-

Pomegranate: India is largest producer of Pomegranate in the world. Variety of Pomegranate are having soft seed less acids and attractive colours of fruits and grains. India can export Pomegranate throughout the year. It is being produced in the states of Maharashtra and Karnataka which are close to the western port of Mumbai. Cooperative society of Maharashtra have formed an apex cooperative in the name MAHA ANAR. Farmers of this area are trained in the production and registers with GLOBAL GAP certification. The total export of Pomegranate was 30158 tonnes with a value of 14726 lakh in 2011-12 (APEDA Website, accessed on 10 July 2015). The main varieties being exported are Ganesh, Rubi, Arakta and Bhagwa. The major countries where Pomegranate exported are UAE, Saudi Arab, Kuwait, UK, Russia and Thailand.

Fig. 4. Corrugated fibre board (CFB) Boxes for Pomegranate (APEDA, 2005, New Delhi).

Carbboard corrugated boxes of 4-5 kg capacity with a dimension of 375*275*100mm and 480*300*100mm are the sizes of boxes respectively as shown in fig.4. These are made up of 3 to 5 ply and have a bursting strength of minimum 10 kg/cm^2. Puncture resistance is minimum 250 ozs inches/tear inch Min and compression strength 150. kgf ,Min. Burst factor (Craft) minimum20, manufacturing joint should be made by glue and with number of ventilation 16 at different angles of the box. Number of pieces/box should not be more than 2.

Measures for enhancing competitiveness for export

- There is a need to export Pomegranate to South East Asian countries to economise the cost of transport and popularise in Canada, USA, south America Australia, Korea and Japan.
- There is a need of packing house in the major growing area in Karnatka and Andhra Pradesh.

Litchi: India is second largest exporter of Litchi in the world. India produces superior Litchi varieties having high pulp to stone ratio and with high yields. The total production of Litchi is concentrated mainly in Bihar, West Bengal, Assam and Jharkhand and to a smaller extent in Tripura, Punjab, Uttarakhand and Orissa. India is in advantageous position with regard to geographical location compared to Thailand and China, as India is nearer to Europe and Gulf countries for exporting Litchis to these countries. Export of Litchi from India during 2014-15 is 961.43 tonne which value 215.18 lakhs (APEDA Website, accessed on 10 July 2015). Litchi variety mainly Shahi, Early Bedana, Late Bedana and Bombai are exported. Uttarakhand farmers they have also framed a society Litchi Exporters Association, Nainital, which is major exporter of Litchi.

Fig. 5. Corrugated fibre board (CFB) Boxes for Litchi (APEDA, 2005, New Delhi)

Packaging is normally done in corrugated or solid fibre board cartons as shown figure 5. Normally Corrugated Fibre Board boxes of capacity 2 kg and 4 kg are used for export with bursting strength of 6 and 10 Kg/cm^2 and puncture resistance and compression strength of 225 and 350 Kgf.

Measures for enhancing competitiveness for export:

- To exploit export of organic litchi in foreign markets.
- Assam and Punjab needs to be encouraged and to facilitate pack houses.

Pineapple: Pineapple is cultivated in India in North East, West Bengal and Eastern Bihar. Western ports can be exploited for exporting to Gulf and European Union which will save a transport also. Agri Export Zone for promoting exports of Pineapple has already been established in North Bengal. There are good prospects for cultivation of organic Pineapples in Kodagu district of Karnataka and Ratnagiri district in Maharashtra state. Total export of Pineapple in 2014-15 is 3751.53 tonne with value of 1664.92 lakhs (APEDA Website, accessed on 10 July 2015) The most common varieties exported from India Kew, Queen and Mauritius. Pineapple is mainly transported by ship and to small extent by air.

Fig. 6. Corrugated fibre board (CFB) Boxes for Pineapple (APEDA, 2005, New Delhi)

Packaging is normally done in corrugated or solid fibre board cartons as shown figure 6. Four sizes of pineapple are packed in different packaging 1.5kg (A), 1-1.5kg (B.), 0.6-1 kg (C) and 0.5 Kg (D). A single vertical pack contain 4 fruits in 2-3 layer and small sized 5 fruits in 5 layers are packed. Dimensions of boxes 535*290*280 and 535*430*195. Partitions in the box are done after each layer with corrugated fibre board. Number of holes in the box will depends upon the number of fruits i.e. 1. (Post- Harvest Manual for Export of Pineapples, APEDA, New Delhi)

Measures for enhancing competitiveness for export:

- To save on transport, advantage of western ports needs to be taken for exporting to Gulf and European countries and to meet the huge demands of European Union (EU), South and Central American countries.
- Need for packhouses and cool chain facilities.

Banana: Banana is an important fruit crop of many tropical and subtropical regions of India. Largest area under Banana cultivation is in Tamil Nadu state followed by Maharashtra, Gujarat, Andhra Pradesh and Karnataka states. Grand Naine, Robusta, Dwarf Cavendish, Nendran and Red Banana are variety commonly grown in India. Export of Banana during 2014-15 is 63274.40 Tonne which value 24194.77 Lakhs (APEDA Website, accessed on 10 July 2015).

Fig. 7. Corrugated fibre board (CFB) Boxes for Banana (APEDA, 2005, New Delhi)

For packaging bananas, boxes of 5 ply strength and of the following dimensions need to be used- Card board fibre boxes and other materials-

- Top = 48.25cm X 31.75cm X 20.25cm -5 ply
- Bottom= 47.50 X 31.25cm X 19.75cm -5ply
- Gap plate= 3 ply

Foam sheet or foam pad= 20mm thick, 38cm X 25cm size with 10 mm holes. Weight of final packed box is approximately 13.0 Kg (Fig. 7)

Measures for Enhancing Competitiveness for Exports:

- Production technology on modern lines needs to be demonstrated to the growers.
- Farmers should be educated for export requirements and international quality standards.
- Protocol for shipping to Gulf countries need to be standardized.
- Most modern packhouse facilities need to be created in Maharashtra and Gujarat.
- It will be advisable to have some working arrangements for ripening of our banana.

Mango: Indian mangoes come in various shapes, sizes and colours with a wide variety of flavour, aroma and taste. The Indian mango is the special product that substantiates the high standards of quality and bountiful of nutrients packed in it. A single mango can provide up to 40 percent of the

daily dietary fibre needs – a potent protector against heart disease, cancer and cholesterol build – up. In addition, this fruit is a warehouse of potassium, beta - carotene and antioxidants. In India, mangoes are mainly grown in tropical and subtropical regions. The major mango-growing states are Andhra Pradesh, Uttar Pradesh, Karnataka, Bihar, Gujarat and Tamil Nadu. Andhra Pradesh ranks first in mango production with a share of 24.48% and highest productivity. India is also a prominent exporter of fresh mangoes to the world. The country has exported 36329.01 metric tonnes of fresh mangoes to the world worth Rs. 317.10 crores during the year 2015-16 (Gandhi, 2016). The main varieties being exported are Ganesh, Rubi, Arakta and Bhagwa. The major 5 importing countries of India's Mangoes were UAE, Bagladesh, UK, Saudi Arabia, and Nepal respectively; these countries alone comprises of around 87% of India's total export of Mango.

Domestic strengths for exporting mango from India are listed below:

- Agri Export Zones for facilitating exports have been established in almost all mango growing areas.
- Packhouses on modern lines have been provided in all mango exporting regions i.e. in Ratnagiri and Sindhudurg in Maharashtra and in Navsari and Borsad in Gujarat for Alphonso variety; in Latur and Aurangabad for Kesar mango; in Saharanpur and Malihabad in U.P. for Dashehari and Chausa mangoes.
- Facilities for facilitating mango exports like Post-harvest Management Centre have been established at Malihabad and Saharanpur. Similarly a mango Export Facility Centre has been established at Ratnagiri.
- Mango farmers of Alphonso and Kesar are already being trained in GLOBALGAP requirements.
- Mango growers of Saharanpur have already branded their product as "NAWAB" mango.
- Facilities for Vapour Heat Treatment and irradiation for eliminating fruit fly have already been set up.

Fig. 8. Corrugated fibre board (CFB) Boxes for packing of Mango (APEDA, 2005, New Delhi)

As shown in fig. 8 fruits are packed in cardboard boxes of 3 and 5 ply with a dimensions of 230*140*140 and a capacity of 4 and 8 kg, bursting strength of 6.5 and 10 kg/cm^2 respectively, compression strength of 275 kgf and with 4 holes (Post- Harvest Manual for Export of Mangoes, APEDA, New Delhi.).

Measures for Enhancing Competitiveness for Exports:

- Fruits shall be packed in compliance with recommended International code for packaging of fresh fruits (CAC/RCP 44-1995, Amd. 1-2004).
- Creation of CA facilities (helpful to keep the fruits for long time) on large scale will help to increase export potential of all fruits.

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A report on Horticulture Statistics Division, DAC&FW

RESEARCH ARTICLE

IN-SILICO CHARACTERIZATION AND HOMOLOGY MODELING OF PEPCK ENZYME OF *MEDICAGO TRUNCATULA*

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Abstract: Phospho*enol*pyruvate carboxykinase (PEPCK) is an enzyme in the lyase family. PEPCK is an ATP-dependent that is involved in the metabolic pathway of gluconeogenesis. It converts oxaloacetate into phospho*enol*pyruvate and carbon dioxide. In this study, the results of structural and physiochemical study of *Medicago truncatula* PEPCK has explored. The conceptual three-dimensional structure investigated while there was no structural information available in any other database. Computational analysis performed on *Medicago truncatula* PEPCK and developed a three-dimensional structure of PEPCK enzyme using comparative modeling approach. The modeled enzyme includes N-terminal and C-Terminal domains with a mixed α/β topology. The energy of constructing models was minimized and the quality of the models was evaluated by VERRIFY_3D and PROCHECK. Ramachandran plot analysis showed the confirmation of 100 % amino acid residues was within the most favored regions. Multiple sequence alignment of the PEPCK protein sequence of different plant sources revealed the conserved region and constructed a phylogenetic tree. The stability of model checked through Gromacs 4.5. The final three-dimensional structure submitted in the protein model database (PMDB). This study may play keystone role in in-vivo and in-vitro studies.

Keyword Phospho*enol*pyruvate carboxykinase, phylogenetic tree, Gromacs, MD simulation, Homology Modeling

INTRODUCTION

Phospho*enol*pyruvate carboxykinase (EC: 4.1.1.49) is an inducible enzyme, which is found in plant, animal and microorganism cells (Walker *et al*., 2002). PEPCK is a cytosolic enzyme that catalyzes the reversible reaction in plants (Malone *et al*., 2007):

$$OAA + ATP \leftrightarrow PEP + ADP + CO_2$$

PEPCK occurs in phloem companion cells, roots, the flesh of fruits, stomata guard cells, simple and glandular trichomes, latex-producing ducts, developing seeds, germinating seeds and in the leaves of many C4 and CAM (Crassulacean acid metabolism) plants (Borland *et al.*, 1998; Kim and Smith, 1994; Leegood and Walker, 2003; Walker and Chen, 2002). The function of PEPCK has clearly established in some of the plant cell type. In germinating seeds, it catalyzed an essential reaction in the conversion of lipids and some amino acids to sugar by gluconeogenesis (Leegood and Ap Rees, 1978; Rylott *et al.*, 2003). In the leaves of some C_4 and CAM plants, it functions as a decarboxylase in photosynthetic CO_2-concerning mechanism (Burnell and Hatch, 1988; Dittrich et al., 1973). This evidence showed that PEPCK plays a vital role in the metabolism of nitrogenous compound in some other cells and tissues (Chen *et al.*, 2004; Delgado-Alvarado et al., 2007; Leegood and Walker, 2003). The mechanism and regulation of C_4 acid decarboxylation in phospho*enol*pyruvate (PEP) carboxykinase-type C_4 plants examined in isolated bundle sheath cell strands. The ATP is required to derive this reaction and derived from mitochondrial respiration, due almost exclusively to malate oxidation via a NAD-specific malic enzyme. The contribution of photosynthesis was negligible to the ATP consumed by this cytosolic reaction (Carnal *et al.*, 1993).

Protein executes most of the work of the living cell. With the innovation of sequence technology, it is now significantly easier to obtain the uncharacterized function of protein in the organism. Still, there are the protein sequences with their functions yet to be discovered or experimentally confirmed (Miller and Attwood, 2003). *Insilico* analysis help in determining the protein functions, which can be divide into three broad categories: sequence, expression and interaction based methods. Sequence based methods rely on the ability to construct the alignment among the protein sequences (Pellegrini, 2001). The sequence analysis approach was used for the prediction of protein domain family and their identification was based on the amino acid sequence similarity. However, structural analysis and comparative study permit confirming the function of the protein and all these predictions are based on the sequence alignment (Pearson, 2013).

In this study, the theoretical 3D model of PEPCK enzyme of *Medicago truncatula* was constructed in order to elucidate the structural properties. The various computational tools used to validate the model structure, and its stability was checked by Molecular dynamics (MD) simulation.

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MATERIAL AND METHOD

Sequence Retrieval and Template selection

The protein sequence of Phospho*enol*pyruvate carboxykinase enzyme of *Medicago truncatula* was retrieved from NCBI (National Center for Biotechnology Information) (Wheeler et al., 2003) database for modeling, which have 658 amino acid residues and their accession number is XP_003612927.1. Comparative modeling usually starts by searching the PDB (Protein Data Bank) (Berman et al., 2000) of the known protein structure using the target sequence as a query. This search is generally done by comparing the target sequence with the sequence of each structure in the database. The protein sequence of Phospho*enol*pyruvate carboxykinase (PEPCK) of "*Medicago truncatula*" used as a target sequence. For identification of suitable template, BLASTp (Johnson et al., 2008) was performed against PDB, and the template was selected for generating the putative 3D structures.

Secondary structure analysis

Secondary structure of PEPCK protein comprises mainly of coils, sheets and helix. In the present study secondary structure of PEPCK protein was predicted by using PDBsum (De Beer et al., 2014) and SOPMA (Geourjon and Deléage, 1995). The predicted secondary structure helped us to improve the alignment between target and template protein.

Comparative Modeling

The method of comparative modeling requires for the identification of homologous sequences with known structure. The first step of comparative modeling is scanning and selection of template protein structure using the target sequence as query (Berman et al., 2000). BLASTp is generally used for template selection procedure (Johnson et al., 2008). BLAST results output potential template for modeling is 1II2, and the high degree of primary sequence identity was 49.40% between Phospho*enol*pyruvate carboxykinase of "*Medicago truncatula*" (target) and *Trypanosoma cruzi* (template) indicates the crystallographic structure that was good model to be used as template for PEPCK enzyme. The alignment of the *Mt*PEPCK target and *Trypanosoma cruzi* (PDBID: 1II2) is shown in Fig. 1. Fifty models were constructed through MODELLER 9v10 (John and Sali, 2003). MODELLER implements comparative protein modeling by satisfaction of spatial restraints.

Analysis of the model

The several models generated through Modeller for the same target and the best model selected for further analysis. We evaluated the model with the lowest value of the Modeller objective function and PROCHECK statistics (Laskowski et al., 1993). The overall stereo-chemical quality of the models for PEPCK enzyme checked through Ramachandran's map calculation it was computed through the Structure Analysis and Verification Server (PROCHECK and VERIFY-3D) program that is available on Naional Institute of Health (NIH) server (http://services.mbi.ucla.edu/SAVES/) (Eisenberg et al., 1997).

Visualization and structural analysis

Visualization and Structural analysis of the final protein model was carried out through PyMol software (DeLano, 2002). 3dSS (Sumathi et al., 2006) and CHIMERA (Pettersen et al., 2004) were used for the structural alignment and RMSD calculation with model and template protein structure.

Active site analysis

After building of protein model, the possible deep cleft active site of PEPCK was explored through CASTp program (Binkowski et al., 2003) (http://sts.bioengr.uic.edu/castp).

Physiochemical characterization:

For physiochemical characterization theoretical pI (isoelectric point), molecular weight, -R and +R (total number of positive and negative residue), EI (extinction coefficient), II (instability index), AI (aliphatic index), and GRAVY (grand average hydropathy) were computed using the Expasy's ProtParam server for set of proteins (http://us.expasy.org./tools/protparam.html), and the results are shown in Table 2.

Multiple sequence alignment and Phylogenetic analysis

The amino acid sequence of M. *truncatula* PEPCK and their all identified homologs were subjected to multiple sequence alignment for recognition of conserved residues and pattern using CLUSTRAL-W program implemented in MEGA-5 program (Tamura et al., 2011). Phylogenetic analysis is a method to find the evolutionary relationship between the species using their protein sequences. The NJ method implemented in MEGA-5 program was used to construct the phylogenetic tree.

Molecular Dynamics Simulation of PEPCK

MD simulation was performed through GROMACS (GROningen MAchine for Chemical Simulation) (Van Der Spoel et al., 2005) package, with Gromacs 96.1 (43A1) force field. The accurate force field is necessary for reproducing the conformational and dynamic behavior of condensed-phase system. The Gromos 96.1 force field has well parameterized for protein. For solvate the model, it has placed in a cubic box maintaining a distance of 1.5 nm between the box edges and the protein surface. Particles mesh Ewald (PME) electrostatic and periodic boundary conditions applied in all directions. The system was neutralized by adding six Na+ counter ions since the overall protein charge was negative. To get rid of the high-energy interactions and steric clashes of the system, the energy of the system minimized using the steepest descent method until a tolerance of 1000 KJ/mol. All the bond lengths were constrined with the LINear Constrains Solver (LINCS) (Hess et al., 1997) method, whereas the geometry of water molecules was constrained with SETTLE algorithm

(Miyamoto and Kollman, 1992). The energy minimization system treated for 200 ps at constant temperature (300 K), pressure (1 atm) and without any position restraints. All the analysis of MD simulation was performed using XMGRACE software (Hartmann, 2009). The best model for further analysis selected at 1000 ps MD simulation and the structure has low RMSD value.

Submission of the modeled protein in protein model database (PMDB)

The generated model of *Medicago truncatula* PEPCK protein submitted successfully in Protein model database without any stereochemical errors. Submittion id of the model is PMID: PM00779121.

RESULT

Physiochemical characterization

Phospho*enol*pyruvate carboxykinase protein (72.475 Kda) of *Medicago truncatula* was extracted from Genbank. Glycine 8.20 and Tryptophan 1.22% (fig. 1) was identified the most and the least number of amino acids in the sequence. shown

The primary sequence analysis PEPCK enzyme is illustrated in Table 2 since, the isoelectric point (pI), solubility is minimized and mobility of an isoelectro focusing system is zero due to calculated pI will be useful. Isoelectric point (pI) is the pH at which the surface of the protein covered by the charge, but the net charge of the protein is zero. At pI, protein is stable and dense. In processing of buffer system, purification by isoelectric focusing method the computed isoelectric point (pI) will be valuable. While Expasy's Protparam computes the extinction coefficient of 276, 278, 279, 280 and 282 nm wavelengths, 280 nm has elected since protein absorbed light strongly. Extinction coefficient of PEPCK enzyme of *Medicago truncatula* at 280 nm was 77280 M^{-1} cm^{-1}. The computed extinction coefficient can help in the quantitative study of protein-protein and protein-ligand interaction in solution. The instability index provides and determines of the stability of protein in a test-tube. There are definite dipeptides and the occurrence of which is particularly divergent in the unstable protein compared with those in the stable once. This method assigned a weight value of instability, which is feasible to compute an instability index (II). A protein whose instability index is slighter than 40 is estimated as stable and a value above 40 estimates that the protein may be unstable. The instability index of PEPCK enzyme of *Medicago truncatula* was found 35.20, which indicates that the protein is stable. The aliphatic index (AI) is elucidated as the relative volume of a protein occupied by aliphatic side chains (A, V, I and L) that is estimated as a positive factor for increase of the thermal stability of globular proteins. Aliphatic index (AI) for the PEPCK enzyme was 75.91. A very high aliphatic index of the protein sequence indicates that the protein may be stable for a vast temperature range. The minimal thermal stability of protein was indicative for a more flexible structure then compared to other protein. The Grand Average hydropathy (GRAVY) value for a peptide or protein is calculated and the sum of hydropathy values of all the amino acids, divided by the number of residues in the sequence. A GRAVY index of PEPCK enzyme was -0.395. This low value shows the probability of better interaction with water.

Sequence analysis

The accession number of retrieved sequences along with the organism's name is listed in Table 1. The multiple sequence alignment was performed for nine plant species (Table 1) using ClustralX with Bioedit. The tool is used with default parameters, and results showed the presence of some conserved regions among all the sequences from different sources and only in some regions have a point different that can environmental effect such as mutation.

Besides, the sequence similarity among these enzymes is shown in Table 3 and the conserved protein portion or residues with their respective positions in *Medicago truncatula* PEPCK (fig. 3) and other organism's sequences from residues 110 to 145, 180 to 270 and from 272 to 660. The phylogenetic analysis was used through MEGA 6 software, and the phylogenetic tree constructed using NJ plot (fig. 4). The results revealed that the PEPCK protein *L.alabamica* of Brassicaceae family was closely related to *A.thaliana*, *P.trichocarpa* and *M.truncatula* while *M.notabilis*, *N.tabacum and S.lycopersicum* are closely related, and these two clusters were distantly related to *Z.mays* and *C.sativus* (fig. 4).

The secondary structure analysis showing 39.06% amino acid residues involved in random coil formation it is higher and 7.90% amino acid residues involved in β-turn formation. Others 30.55% residues involved in α-helix formation and 22.40% residues involved in extended strand formation (Table 4). According to topology analysis, 27 β-strands and 19 α-helixes were found (fig. 6) and fig. 4 showing the α-helix, β-strands and coils position in the structure.

Comparative Modeling

Comparative modeling of protein provides a significant hypothesis of homology between the target and template. This approach provides reasonable results based on the assumption that the tertiary structure of the two proteins will be similar if their sequences are related. Absence of the experimentally determined three-dimensional structure of Phospho*enol*pyruvate carboxykinase protein of *Medicago truncatula* is in experimentally proved database (Protein Data Bank). Comparative modeling method was utilized to construct its theoretical three-dimensional structure. BLAST (Basic Local Alignment Search Tool) scanning results revealed more identical with carystallographic structure *Trypanosoma cruzi* (PDBID: 1II2 with 2.0

Å resolution) while the template was determined on the basis of higher sequence identity. Sequence identity is 49.4% with 330 conserved residues and 66.5% sequence similarity. Comparative modeling predicts the three dimensional structure of the hypothetical model of a given protein sequence (target) based primarily on this alignment to the template. The resulting 3-D structure of Phospho*enol*pyruvate carboxykinase was sorted according to the score calculated from discrete optimization protein (DOPE) scoring function. The final model was selected for further study (fig. 7), which has the lowest root mean square deviation (RMSD) and relative to the trace of the crystal structure.

Model quality assessment

The detailed residue-by-residue stereo-chemical quality of the modeled protein structure was evaluated by the Ramachandran plot (fig. 8) using procheck tool. The reliability of the backbone torsion angle Φ and Ψ distribution of the protein and the template was evaluated by the Ramachandran plot in Procheck tool. The received Ramachandran plot (Phi-Phi) pairs had 84.0% residues in the most favored regions, 13.20% core residues in additional allowed regions, 0.90% residues generously allowed regions and 1.30% residues in disallowed regions Table 5. This value indicates a good quality model, whenever the crystal structure of *Trypanosoma cruzi* (PDB ID: 1II2) shows 89.70% residues in most favored regions. In order to characterize the model, structural motif and mechanically important loops were assigned to build a final 3D model of PEPCK protein. The packing quality of each residue of the model was assessed by Verify_3D program where the compatibility of the model residue with their environment is assessed by score function. Residue with a 3D-1D score of >0.2 should be considered reliable. As shown in Table 5 the score of the refined model is 68.24% of the residues have an averaged 3D-1D score. ProSA revealed a Z-score of -5.85 (fig. 9) for modeled protein.

RMSD (root mean square distance) between the equivalent Cα atoms pair (target and template) was measured to check the degree of structural similarity. The best-modeled structure for fitting into the template (crystal structure) was examined, the prepare model and its closest relative were superimposed based on Cα and backbone atom pairs. Pairwise 3D alignment search of the template protein with the modeled structure through 3dSS and CHIMERA showed the massive RMSD of 0.259 Å (fig. 10) on their backbone atom. These server's results conclude that PEPCK protein and its homologues share strong structural conservation and similarity in the structural folding. It signifies that the generated model is reasonably good for further studies. The final model was scanned through Castp with 1.4 Å probe radius, it was showed 12415 volume of the cavity with 4242.8 area. The amino acid residue involved in the formation of cavity shown in (fig. 11).

Molecular dynamics Simulation of PEPCK enzyme

MD simulation using Gromacs 4.5 confirmed the high stability of the selected model. Energy minimization was performed using the steepest descent methods. The RMSD and RMSF were found to be optimum as per the Gromacs manual and are given graphically. The steepest descent energy minimization for the solvated modeled protein revealed the maximum force reached the threshold of 1000 $kJ^{-1}nm^{-1}$ in 400 steps. The RMSD of the protein backbone atoms are plotted as a function of time to check the stability of the system throughout the simulation. Compared to the starting coordinates, the RMSD of the backbone atom increased in the first 500 ps and then reached a plateau in the subsequently simulation time (fig. 12). The relative flexibility of the model was also characterized by plotting the root mean-square flexibility (RMSF) related to the average structure obtained for the MD simulation trajectories. Three flexible regions have predicted for the modeled protein structure of PEPCK considering the RMSF value and represented in (fig.12). One flexible region has been located in the N-terminal end, and the RMSF value of the region is 0.92. The second flexible region is nearby middle region of the protein, and this region processes several continuous peaks. The RMSF value of the peaks in this region is 0.62 and 0.59. The C-terminal end has a single flexible region with a 0.45 RMSF value. It can be summarized from the RMSF analysis the middle region of the modeled protein is more flexible in comparison of N-terminal and C-terminal.

CONCLUSION

In this study, both sequence and structural activities of phospho*enol*pyruvate kinase enzyme through comparative genomics and molecular modeling approaches were examined. The aggregation of Bioinformatics tools was focused not only on sequence analysis but also structural information, guided us to suggest the uncharacterized information of phospho*enol*pyruvate kinase (PEPCK) protein of *Medicago truncatula*. The functional information of PEPCK protein suggested the involvement in breaking of various chemical bonds. This type of elimination reaction generates a double bond but not hydrolytic or oxidative reaction. There are two distinct types of feature found in that protein (i) PEPCK-ATP active domain with residue 135 to 603. It catalyses the first committed (rate-limiting) step in the diversion of tricarboxylic acid cycle intermediates toward gluconeogenesis. It catalyzes the reversible decarboxylation and phosphorylation of oxaloacetate to yield phospho*enol*pyruvate and carbondiooxide, using a nucleotide molecule (ATP) for the phosphoryl transfer, and has a strict

requirement for the divalent metal ion for activity. PEPCK consist of N terminal and C terminal domain, with the active site and metal ion located in the cleft between them. Both domains have α/β topology that is partly similar to one another. (ii) AAA (ATPases Associated with diverse cellular Activity) domain, they share common conserved module residues 350 to 377 amino acids. This domain belongs to functionally diverse protein family of ring shaped P-loop NTPases, which exert their activity through the energy dependent remodeling or translocation of macromolecule. AAA protein couple chemical energy provided by ATP hydrolysis to conformational changes, which are transduction into mechanical force exerted on a molecular substrate. The secondary structure analysis through SOPMA showing random coils percentage is very high 39.06%, β-turns having lowest percentage 7,90% and α helix having 30.55% whereas Extended strands having 22.49%. Secondly, a 3D model of PEPCK protein using the comparative modeling approach was constructed. The physiochemical characterization of protein by different parameter like as isoelectric point, molecular weight, total number of positive and negative residues, extinction coefficient, instability index, aliphatic index and grand average hydropathy (GRAVY) was determined. The PEPCK protein model had a stable confirmation in response to the atomic flexibility. Computational analysis used to distinguish its structural impulsive feature and validated by the SAVES. The structure-structure alignment between the best-constructed model and template (crystallographic structure) was performed. The calculated RMSD value is 0.259Å and submitted this structure into Protein Model Database (PMDB) and PMDB-ID is PM0080219. In last, we will conclude that this study will be helpful for the further experimental analysis of PEPCK.

Competing Interests

The authors declare that they have no competing interests.

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mtPEPCK         140 NLSPAELYEQAIKYEKGSFITSNGAMATLSGAKTGRSPRDKRVV-KDKVT    188
                    ||...||.:.|:|.||.|.:|:.||:|.:|.|||||||.|||:| .|.|.
1II2_A            7 NLLSPELVQWALKIEKDSRLTARGALAVMSYAKTGRSPLDKRIVDTDDVR     56

mtPEPCK         189 ENELWWGKGSPNIEMDEETFMVNRERAVDYLNSLDKVFVNDQFLNWDPEN    238
                    ||..|   |..|:::.||:|...|:.|.::|::.:.:||.|.|...|...
1II2_A           57 ENVDW---GKVNMKLSEESFARVRKIAKEFLDTREHLFVVDCFAGHDERY    103

mtPEPCK         239 RIKVRIVSARAYHSLFMHNMCIRPSPEELENFGTPDFTIYNAGKFPCNRF    288
                    |:|||:.:.|.||:|||.:|.|.|:||||..||.||:.|||||:...:..
1II2_A          104 RLKVRVFTTRPYHALFMRDMLIVPTPEELATFGEPDYVIYNAGECKADPS    153

mtPEPCK         289 THYMTSSTSIDLNLARREMVILGTQYAGEMKKGLFSVMHYLMPKRQILSL    338
                    ...:||:|.:.||...||.|||||:||||||||:.:||..|||:...|.:
1II2_A          154 IPGLTSTTCVALNFKTREQVILGTEYAGEMKKGILTVMFELMPQMNHLCM    203

mtPEPCK         339 HSGSNMGKGGDVALFFGLSGTGKTTLSTDHNRYLIGDDEHCWSENGVSNI    388
                    |:.:|:||.|||.:|||||||||||||.|.:|.|||||||.|::.||.||
1II2_A          204 HASANVGKQGDVTVFFGLSGTGKTTLSADPHRNLIGDDEHVWIDRGVFNI    253

mtPEPCK         389 EGGCYAKCIDLSREKEPDIWNAIRFGTVLENVVFDEHTREVDYSDKSVTE    438
                    |||||||.|.|:.:.|.||::|:|||.|.||.|.|:.|.|:|:.|:|:.:
1II2_A          254 EGGCYAKAIGLNPKTEKDIYDAVRFGAVAENCVLDKRTGEIDFYDESICK    303

mtPEPCK         439 NTRAAYPIEYIPNAKLPCVGPHPKNVILLACDAFGVLPPVSKLTLAQTMY    488
                    |||.|||:.:|..|....:..||||||.|..|||||:|||::||.||.|:
1II2_A          304 NTRVAYPLSHIEGALSKAIAGHPKNVIFLTNDAFGVMPPVARLTSAQAMF    353

mtPEPCK         489 HFISGYTALVAGTE-DGIKEPQATFSACFGAAFIMLHPTKYAAMLAEKME    537
                    .|:.||||.|.|.| .|.:..:..||:|||..|::.|.|.|...||||:
1II2_A          354 WFVMGYTANVPGVEAGGTRTARPIFSSCFGGPFLVRHATFYGEQLAEKMQ    403

mtPEPCK         538 SHGATGWLVNTGWSGGSYGTG-NRIKLSYTRKIIDAIHNGSLLGAEYKKS    586
                    .|.:..||:|||::||....| .|:.|..||.||||||:|:|...||::.
1II2_A          404 KHNSRVWLLNTGYAGGRADRGAKRMPLRVTRAIIDAIHDGTLDRTEYEEY    453

mtPEPCK         587 EIFGLQIPTEVEGVPSEILDPINAWSDKNAYNATLLKLGGLFKKNF    632
                    ..:||..|..|..||..:|:|..||.|...:|.|..:|..:|:::|
1II2_A          454 PGWGLHIPKYVAKVPEHLLNPRKAWKDVRQFNETSKELVAMFQESF    499
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Fig. 1. Sequence Alignment of *Medicago truncatula* (XP_003612927.1) with *Trypanosoma cruzi* (PDB ID: 1II2). The alignment was performed using EMBOSS.

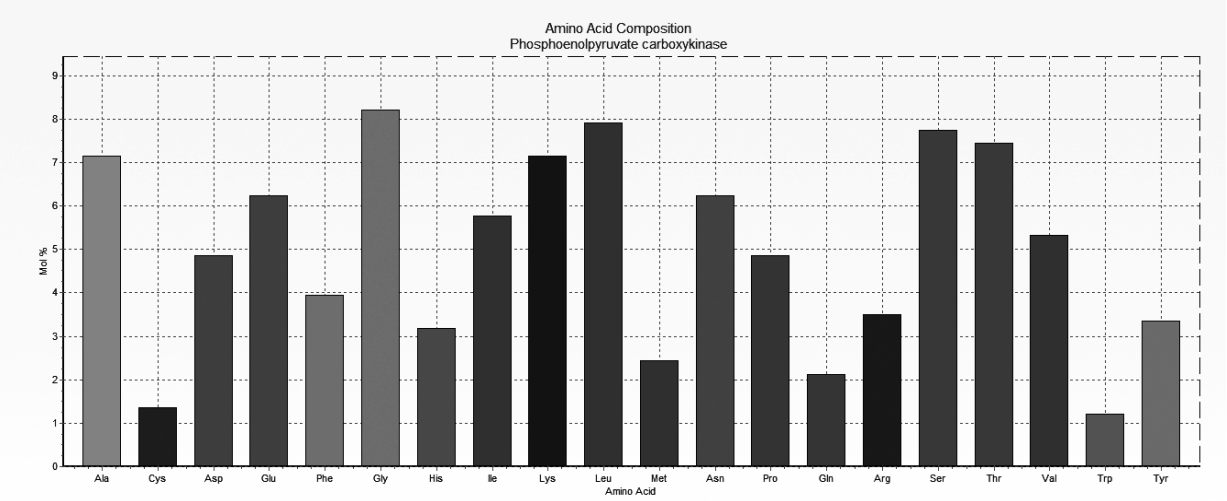

Fig. 2. Amino acid composition in PEPCK enzyme of *Medicago truncatula*

Amino Acid		Site
SLC	TLC	
G	Gly	135, 141, 204, 211, 218, 222, 243, 245, 319, 329, 359, 364, 369, 389, 393, 396, 403, 406, 408, 422, 432, 438, 439, 462, 506, 521, 541, 548, 552, 565, 588, 591, 597, 600, 601, 604, 624, 638, 647, 674, 703
A	Ala	125, 128, 192, 198, 212, 214, 219, 263, 295, 297, 328, 363, 399, 442, 458, 490, 500, 516, 519, 544, 547, 558, 562, 566, 567, 577, 581, 589, 620, 629, 701, 702
L	Leu	67, 70, 127, 130, 180, 186, 194, 216, 240, 267, 270, 280, 301, 315, 350, 370, 377, 384, 386, 400, 404, 412, 420, 447, 465, 514, 515, 523, 529, 531, 545, 580, 593, 611, 626, 627, 653, 669, 672, 675, 695, 700
M	Met	251, 303, 306, 340, 355, 366, 374, 378, 392, 535, 570, 579, 584
F	Phe	106, 206, 256, 302, 318, 323, 331, 371, 401, 402, 461, 470, 520, 538, 560, 564, 568, 637, 676
W	Trp	241, 242, 282, 428, 456, 592, 598, 659
K	Lys	73, 137,203, 220, 228, 244, 289, 367, 368, 394, 409, 443, 451, 501, 528, 554, 583, 616, 633, 662
Q	Gln	71, 119, 197, 278, 361, 533
E	Glu	133, 193, 196, 202, 250, 253, 261, 314, 316, 354, 365, 425, 437, 450, 501, 528, 554, 583, 616, 633, 662
S	Ser	55, 69, 83, 111, 122, 124, 126, 129, 176, 178, 190, 205, 209, 217, 246, 269, 294, 300, 342, 343, 345, 385, 388, 405, 413, 434, 527, 540, 561, 599, 650
P	Pro	85, 88, 136, 143, 191, 225, 247, 284, 310, 321, 332, 379, 453, 493, 498, 503, 509, 524, 525, 556, 573, 642, 649, 655, 704
V	Val	139, 231, 264, 273, 290, 356, 398, 433, 464, 468, 469, 477, 484, 505, 512, 522, 526, 546, 594
I	Ile	123, 199, 207, 249, 288, 292, 308, 325, 357, 383, 421, 436, 455, 459, 494, 497, 513, 539, 569, 609, 618, 621, 652, 699
C	Cys	307, 333, 427, 440, 444, 504, 517, 563
Y	Tyr	195, 201, 266, 298, 326, 339, 362, 376, 441, 479, 492, 536, 542, 576, 603, 613, 631
H	His	68, 184, 299, 304, 338, 375, 387, 426, 473, 508, 537, 572, 622
R	Arg	115, 132, 229, 260, 262, 291, 296, 309, 335, 352, 381, 475, 489, 608, 615
N	Asn	13, 188, 248, 259, 281, 286, 305, 334, 349, 391, 417, 435, 457, 467, 487, 511, 595, 679
D	Asp	53, 142, 177, 227, 233, 252, 265, 283, 322, 347, 397, 415, 423, 424, 446, 478, 518, 619
T	Thr	39, 63, 87, 131, 183, 208, 215, 221, 236, 255, 320, 324, 337, 341, 344, 360, 407, 410, 411, 414, 463, 474, 485, 488, 534, 543, 549, 559, 590, 596, 614

Fig. 3. Identified conserved residues with single letter code and their respective positions

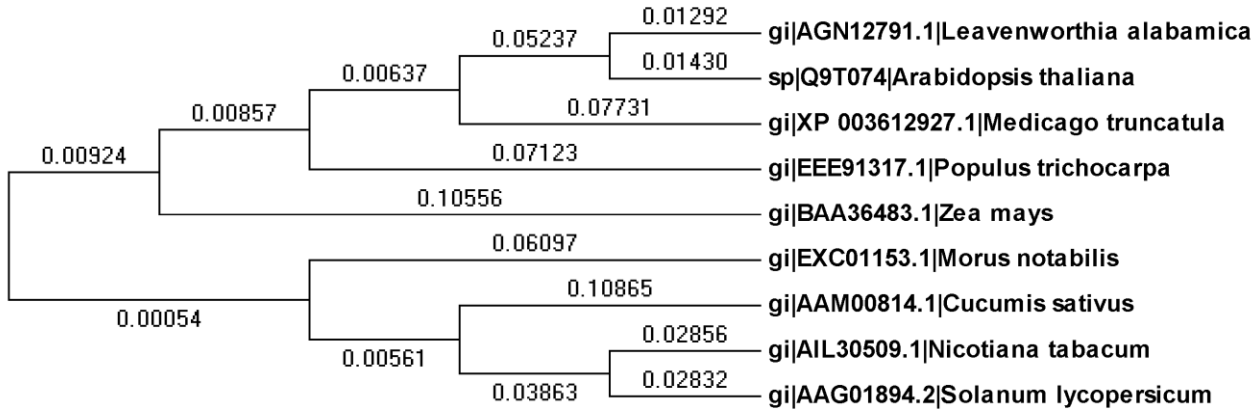

Fig. 4. Phylogenetic tree constructed through MEGA 6 in between different organism.

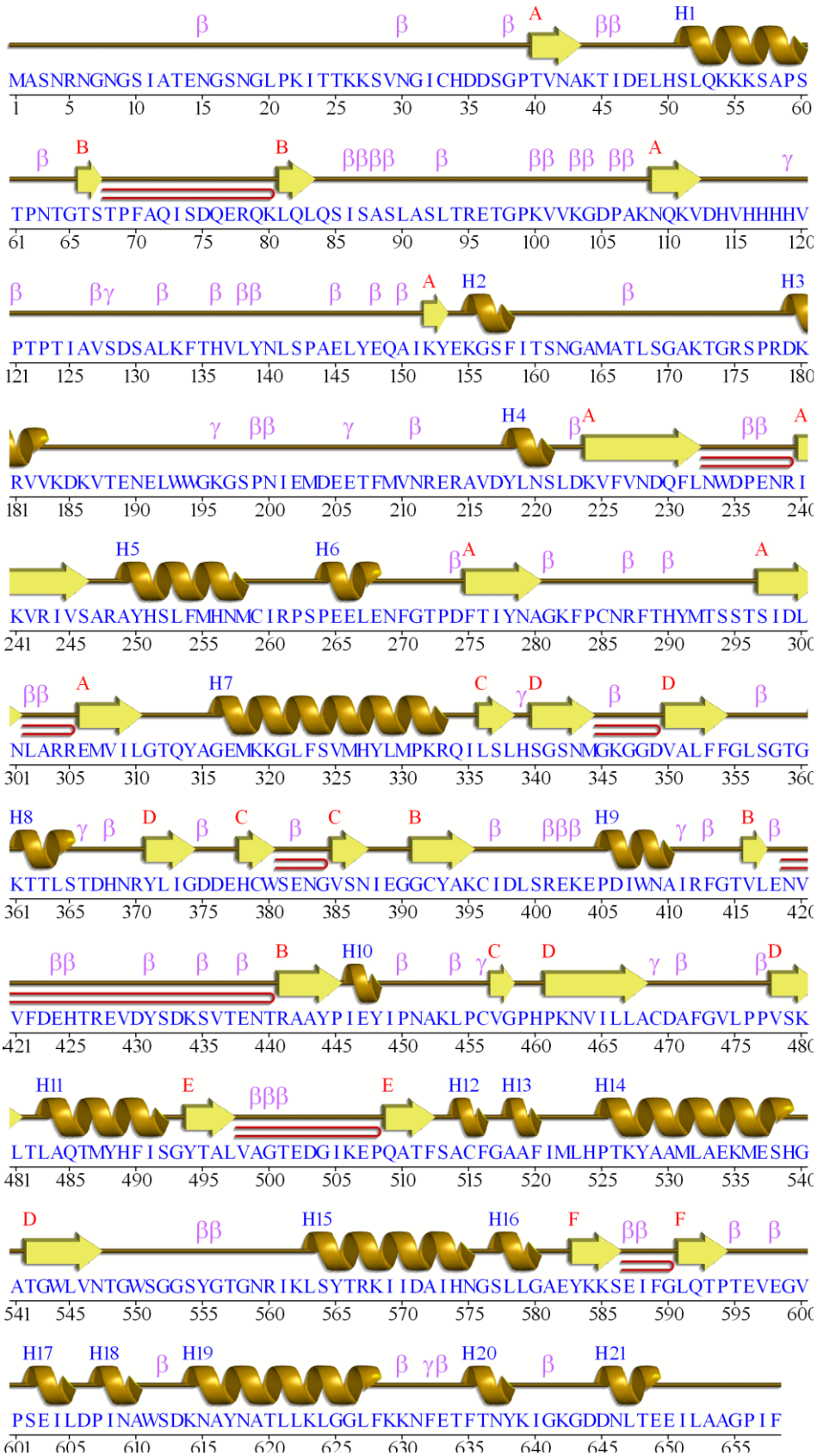

Fig. 5. The graphical representation of PEPCK protein with its secondary structure element

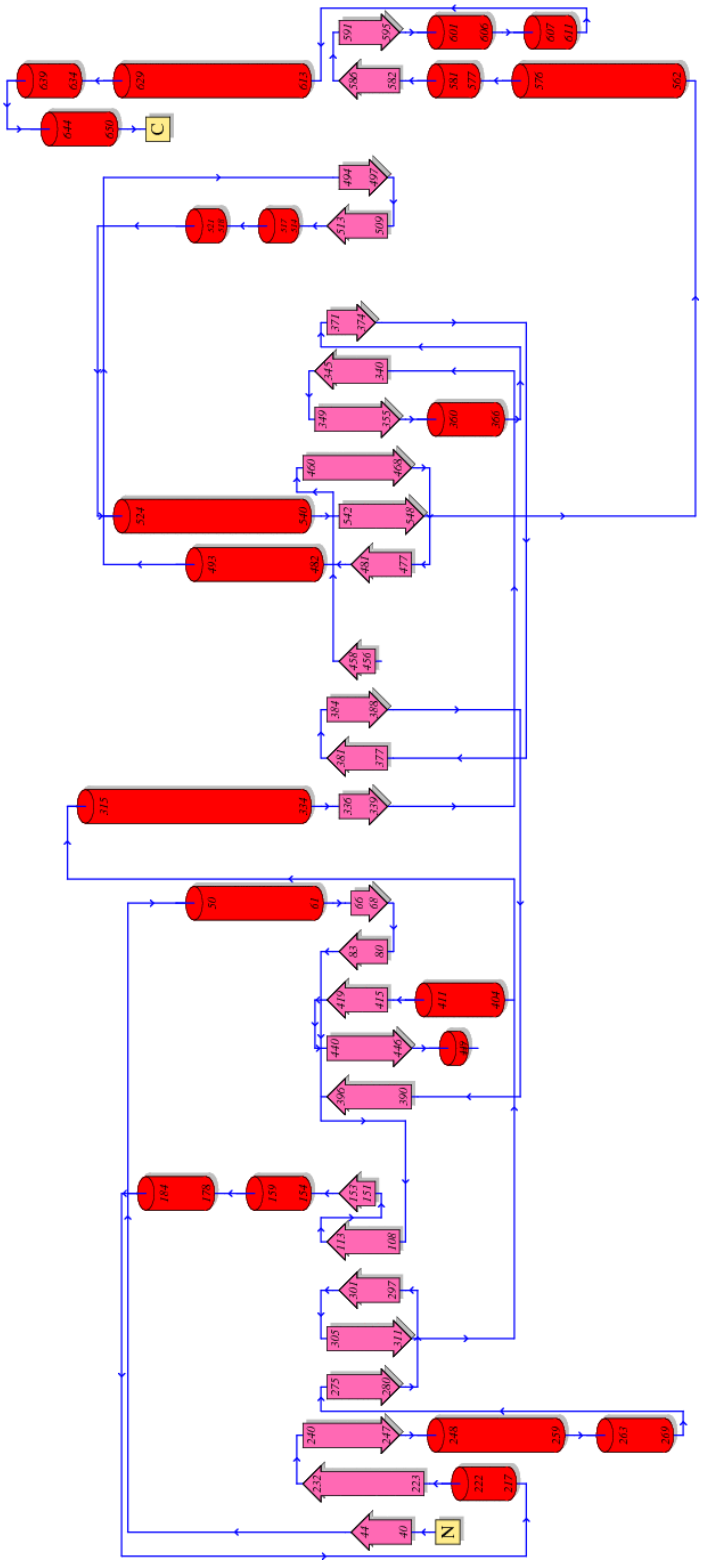

Fig. 6. Topology of predicted structure shown in distinct domains.

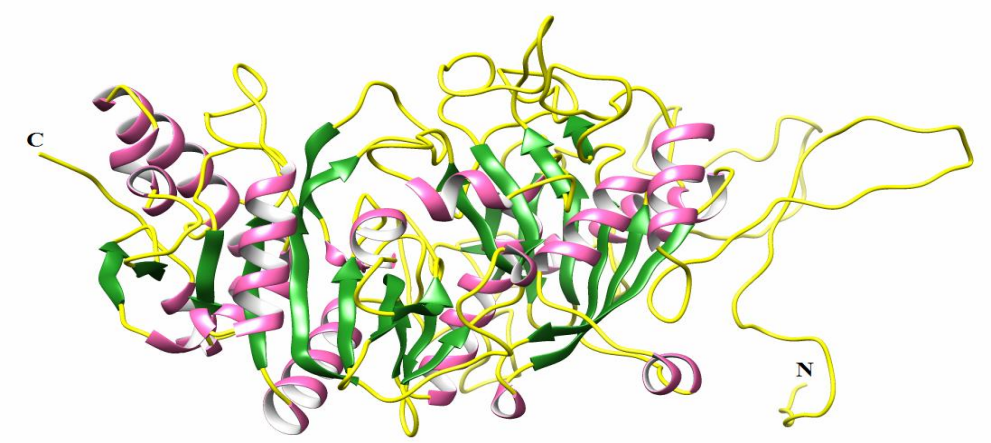

Fig. 7. Homology model of PEPCK protein (NCBI accession: gi|XP_003612927.1) from *Medicago truncatula* solid ribbon representation of hypothetical model colored by its secondary structure element.

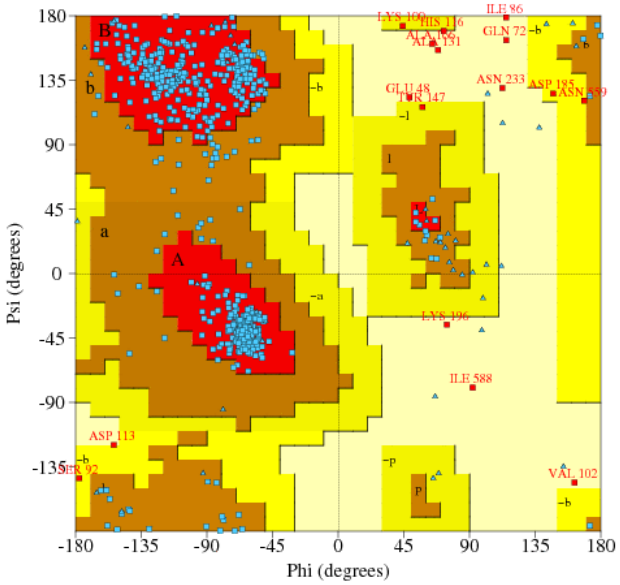

Fig. 8. Ramachandran plot of the modeled PEPCK protein (NCBI accession: gi|XP_003612927.1). The plot was calculated by PROCHECK program

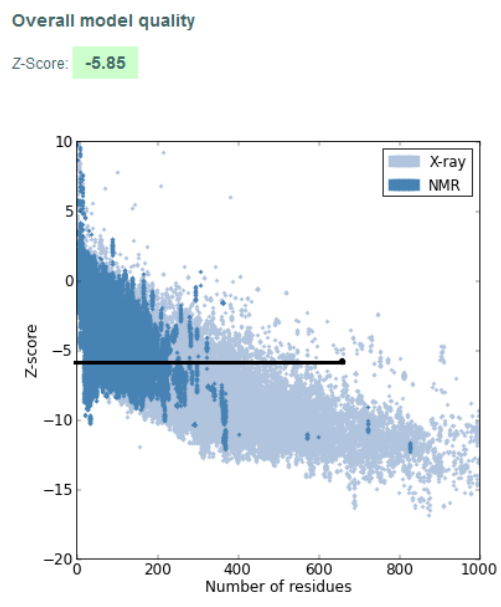

Fig. 9. The Z-score of -5.85 indicated on the graph represent the overall quality of the modeled PEPCK protein.

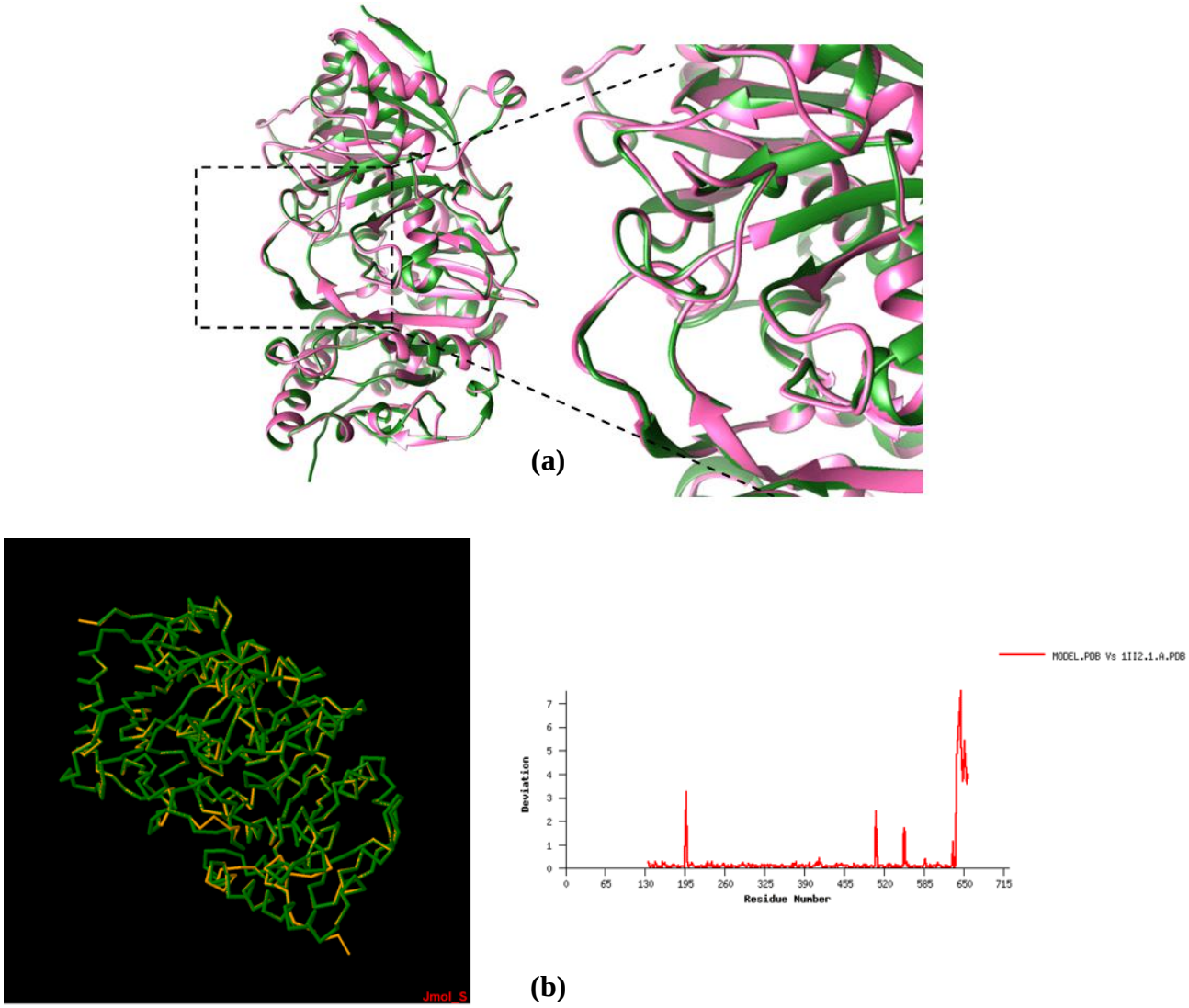

Fig. 10. Superimposition between template and model protein (a) Backbone showing the superimposition of template (*forest green color*) and the refined model (*hot pink color*) and showing the enlarge view of superimposition (b) Showing 3dSS generated image (Jmol view) and graphical representation of RMSD value of amino acid residues of template and model protein structure.

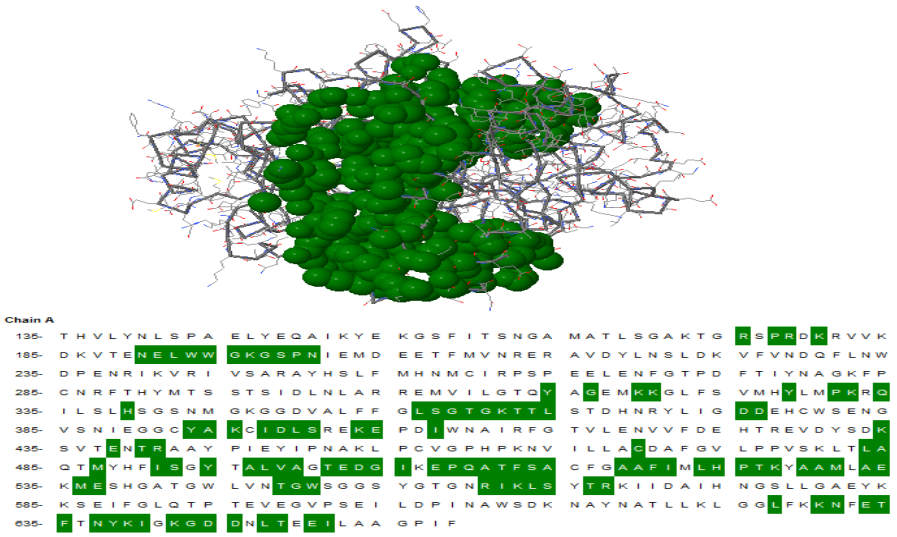

Fig. 11. Pocket identification through castp server highlighted with green color, residues involved in construction of pocket

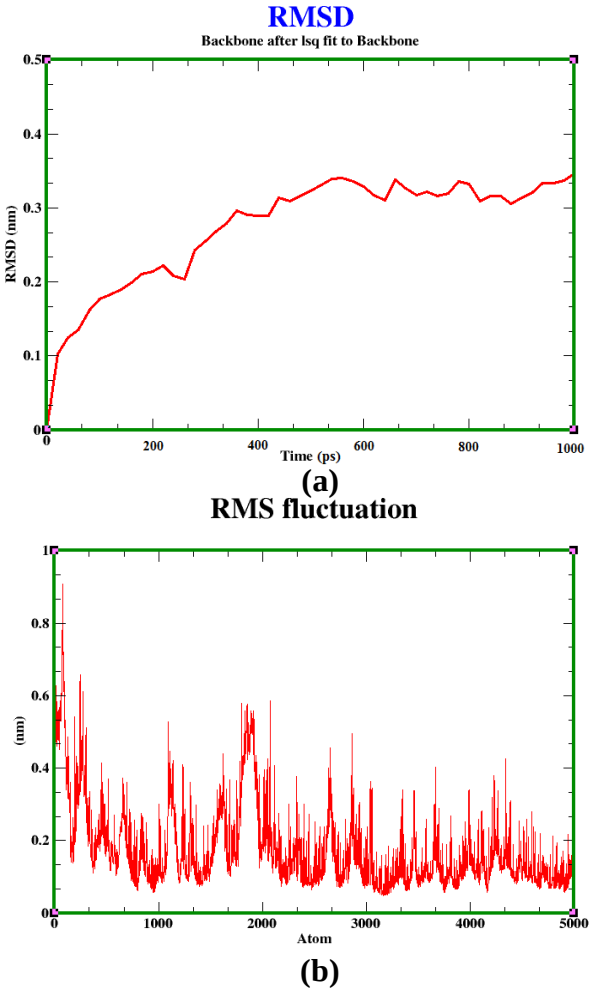

Fig. 12. The RMSD and RMSF graph of the modeled PEPCK protein during MD simulation. (a) RMSD of backbone C atom of the modeled PEPCK protein structure. (b) RMSF analysis of amino acid residues of the modeled PEPCK protein structure.

Table 1. Retrieved sequences from NCBI/Enterz with their accession number

S.No.	Name of organisms	Accession number
1.	*Medicago truncatula*	gi|XP_003612927.1
2.	*Populus trichocarpa*	gi|EEE91317.1
3.	*Cucumis sativus*	gi|AAM00814.1
4.	*Morus notabilis*	gi|EXC01153.1
5.	*Leavenworthia alabamica*	gi|AGN12791.1
6.	*Nicotiana tabacum*	gi|AIL30509.1
7.	*Zea mays*	gi|BAA36483.1
8.	*Solanum lycopersicum*	gi|AAG01894.2
9.	*Arabidopsis thaliana*	sp|Q9T074

Table 2. Physiochemical characteristics computed using Expasy's ProtParam tool

Seq. ID	Seq. Len.	MW	pI	(+)R	(-)R	EC	II	AI	Gravy
XP_003612927.1	658	72475.0	6.73	70	73	77280	35.20	75.91	-0.395
1II2	524	58733.3	8.63	68	63	64330	36.17	78.38	-0.280

Table 3. Multiple sequence alignment-scoring table complied through ClustralW2.

	M.truncatula	*P.trichocarpa*	*C.sativus*	*M.notabilis*	*L.alabamica*	*N.tabacum*	*Z.mays*	*S.lycopersicum*	*A.thaliana*
M.truncatula	-	-	-	-	-	-	-	-	-
P.trichocarpa	83.13	-	-	-	-	-	-	-	-
C.sativus	78.88	77.86	-	-	-	-	-	-	-
M.notabilis	81.46	81.48	80.51	-	-	-	-	-	-
L.alabamica	82.52	81.93	76.27	82.01	-	-	-	-	-

N.tabacum	81.16	82.02	81.87	82.78	82.48	-	-	-	-
Z.mays	79.18	77.86	75.08	79.28	78.53	79.76	-	-	-
S.lycopersicum	82.37	82.18	81.42	83.69	82.18	93.5	80.36	-	-
A.thaliana	83.28	82.38	76.27	81.86	97.02	82.48	78.23	81.87	-

Table 4. Percentage of amino acid sequence forming secondary structure using SOPMA prediction server

S.No.	Name of Enzyme	Accession No.	α helix %	β-turns %	Extended strands %	Random coil %
1	PEPCK	XP_003612927.1	30.55	7.90	22.49	39.06

Table 5. Ramachandran plot statistics with Verify_3D and ProSA

Protein	Most favoured regions	Add. Allowed regions	Gene. Allowed regions	Disallowed regions	Gly residues	Pro residues	Verify_3D	ProSA Z-score
XP_003612927.1	479 (84 %)	73 (13.2 %)	5 (0.9 %)	11 (1.90 %)	54	32	68.24 %	-5.85
1II2	813 (89.7 %)	90 (9.9 %)	3 (0.3 %)	0 (0.0 %)	75	52	-	-

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RESEARCH ARTICLE

STUDY OF SOUTH-WEST MONSOON RAINFALL SCENARIO IN MEERUT DISTRICT

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Abstract: Rainfall is one of the most important climatic variables and renewable natural source of water on the earth. Meerut District is the part of Upper Ganga-Yamuna doaba which lies between 28^0.98' & 29^0.15' north latitude and between 77^0.45' & 77^0.07' east longitude. The objective is to compute properties of a long period of time series is broken into separate components and analyzed individually to understand the pattern of rainfall. The annual and monthly rainfall data used for observed trend during long period. Analysis of rainfall data of a century (1916-2015) over Meerut plays a significant role in the agricultural and urbanization contribution and in the overall growth of the District. The data of annual rainfall and S-W rainfall is 689.6mm and 587.2mm respectively. The monthly south-west monsoon rainfall variability in years is observed maximum after 21th century. It is most important period of rainfall seasonal cycle. The analysis data of S-W monsoon observed highest rainfall 85.2% and lowest rainfall in post monsoon season 4.4%. The anomalous departures from the mean were observed the highest positive and negative departure from the mean of approximately -459.1 & 457.5 in year 2009 and 1933 respectively. The analysis included variability of rainfall, trends in rainfall pattern and changes in spatial and temporal patterns of Precipitation Ratio (PR) and Monsoon Precipitation Index (MPI). The maximum abnormality 2.46 and -2.04 in annual rainfall was recorded during 1933 & 2009. It is seen that the average MPI varied from 0.63 to 0.77. The trend in the annual rainfall showed that the rainfall decreasing in the area whereas south-west rainfall declined pattern of 3% changes was also observed in the century. The standardized anomalies results obtained show a fluctuating rainfall pattern across the years over Meerut District which makes it hard to freely forecast rainfall trend for a future season. The rainfall data analysis of Meerut District for a period of 100 years (1916 to 2015) reveals variation in the rainfall amount and points out a negative trend of rainfall in future. The information is useful for agriculturists and policy makers on critical issues is it affects seasonal agricultural practices such application of agricultural inputs, water resources maintenance and management practices. The global climate and the local environmental changes are the chief factors for the variation in rainfall over the recent times. The knowledge of current situation of weather and climate change related pattern and adaptation of technology is maintend trend. Uncertainty on the dates of monsoon onset and its withdrawal also puts a great problem before the farmers.

Keywords: Anomalies, Climate change, MPI, PR & South-west monsoon

INTRODUCTION

Rainfall is one of the most important climatic variables and renewable natural source of water on the earth. The rainfall patterns have temporal and spatial variability due to seasonal atmospheric phenomenon and geographical factors respectively. The rainfall received maximum coverage area during south west monsoon season: June to September month (Attri & Tyagi, 2010). The variations in rainfall patterns are vital to understand the climate change variations. The variability in rainfall may affect the agriculture production, water supply, transportation, the entire economy of the region, and the existence of its people.

The assessment of climate change is done through statistical analysis of certain meteorological parameters such as annual, seasonal rainfall. Climate change has become big threat for agriculture, livestock, and biodiversity environment. Meerut district has main season in a year (IMD's season). These are: the winter season (January- February.), Pre –Monsoon season (March- May), South West Monsoon season (June-September) and, the Post Monsoon Season (October- December). The south westerly wind flow occurring over most parts of India and Indian seas gives rise to south west monsoon over India from June to September. South-west monsoon provides a major part of India's annual rainfall, and the quantum varies widely across space (GOI, 1999). In most places, growing crops require artificial provision of water during non-monsoon season and in some places even during the monsoon. Weather parameters mainly rainfall, its distribution pattern and quantum play an important role in productivity crops. The prediction of rainfall further helps in planning the activities of agriculturists, water supply professionals or engineers, and others.

Study Area

Meerut District is the part of Upper Ganga- Yamuna doaba which lies between 28^0.98' & 29^0.15' north latitude and between 77^0.45' & 77^0.07' east longitude (Abst. & Souv., 2016). The altitude / elevation (above sea level) of the city are 224.6 m (Fig.1). The

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district is spread across 2564 square kilometer .The land of district is very fertile which is known as alluvial soil or loamy soil deposits by Ganga. Meerut has humid subtropical type climate which is characterized by cool winters and very hot summers. The average annual rainfall of Meerut is about 805.98 mm (Kumar *et al*., 2009). About 80% of the rainfall is received during the south west monsoon (Jain &Kumar, 2012)). The monsoon begin by the end of June and last till the end of September.

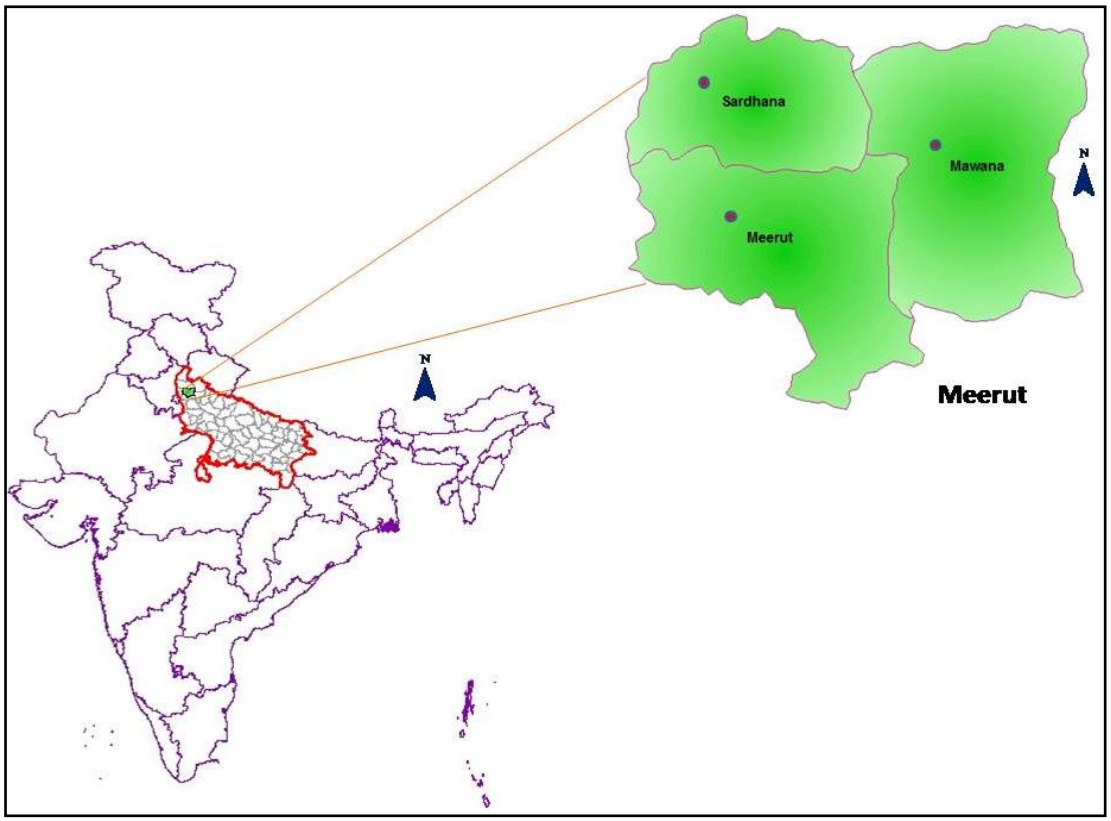

Fig. 1. Study area

Objectives

The objective is to generate data having properties of the observed long period record. To compute properties of a long period of time series is broken into separate components and analyzed individually to understand the pattern of rainfall. The annual and monthly rainfall data used for observed trend during long period.

MATERIAL AND METHOD

The present study is based on secondary sources of time series data (rainfall) data of seasonal rainfall. The climatic data (annual and monthly rainfall) of Meerut for continuous 100 years 1916 to 2015 data were obtained from India water portal website, IMD, New Delhi & NASA POWER (1901-2002, 2003, 2004-06 &2007-13 & 2014-15) (Rainfall data Info: a,b,c,d & e). The data set used in this study is derived from the time series of annual and monthly precipitation of Meerut district, Uttar Pradesh. The S-W rainfall data analysis to observed pattern of trend and develop forecasting model for future scenario. The different type of statistical data analysis *viz.* Coefficient of Variation (CV), Standard deviation, Correlation of Coefficient (R^2), Departure and Cumulative departur**e** and Trend Analysis to given important scenario of change pattern of time series data in MS Excel.

The Following formula has been used for determining Mean, Standard Deviation and Co-efficient of Variation.

(a) Mean $(\bar{x}) = \frac{\sum x}{N}$

Where,

x = rainfall variables, N= number of years

(b) Standard Deviation $(\sigma) = \frac{\sum(x-\bar{x})^2}{N}$

Where,

$\bar{x}$ = the mean value as is defined above.

In computing the deviation score $(\bar{x} - x)$ and the standardized anomaly, formula *viz.*

(c) Standardirzed anomaly $= \frac{(x-\bar{x})}{STD}$

Where,

x is the annual rainfall totals, $\bar{x}$ is the mean of the entire series and

STD is the standard deviation from the mean of the series.

(d) Coefficient of variation $= \frac{\text{Standard Deviation}}{\text{Mean}} x100$

It is also referred to as the coefficient of mean deviation, is defined as the ratio of the standard deviation to the mean of the data set.
(e) Median: It is the middle value when the data is arranged in order of size.
(f) Coefficient of Skewness: The coefficient of skewness measures the skewness of a distribution. It is based on the notion of the moment of the distribution.

$$\text{Skewness} = \frac{\bar{x} - \text{Mode}}{\text{Standard Deviation}} x100$$

Where,
$\bar{x}$ represents the arithmetic mean
This measure is equal to zero if the data are distributed symmetrically.
The Monsoon Precipitation Index (MPI) and Precipitation Ratio have also been worked out using respective formula.

RESULT AND DISCUSSION

The mathematical and statistical analyses of South-west rainfall (S-W rainfall) are discussed in below:

Variation of monthly rainfall

The average monthly rainfall of 100 years (1916-2015) for Meerut district is observed 587.2mm and the intensity of rainfall increasing from June to September (South-west Monsoon), and suddenly decreasing trend noticed from October to December (Post- monsoon). The analysis of 100 years monthly data observed 108.2mm in the month of September, 218.6mm in August 202.6mm in July, and 57.8mm in June. The lowest rainfall was observed 7.3mm in the month of November and its maximum rainfall is 218.6mm in the month of August. The coefficient of variation for monthly mean rainfall observed highest in the month of November and it is 151% whereas coefficient of variation is minimum for the month of August and it is 46.6% for the Meerut district. This shows that rainfall is more stable in the month of August and is more variable in the month of November for the Meerut district. The scenario of seasonal rainfall data observed South-west monsoon has maximum rainfall then other seasonal rainfall (Table-1).

Table 1. Statistical summary of seasonal (month wise) rainfall of Meerut district (1916-2015)

Season	Month	Mean	Std. Dev.	C.V. %	MIN.	MAX.	MEDIAN	COFF. OF SKEWNESS	Distribution % of Rainfall
Winter	January	18.1	15	82.7	0.1	58.3	16.1	0.8	5.1
	February	16.8	17	101.2	0	100.4	12.8	1.8	
Pre-Monsoon	March	13.2	14.9	112.7	0	98	9.2	2.6	5.4
	April	8.8	10.1	115.7	0	44.3	5.0	1.9	
	May	15.5	14.5	93.9	0.2	67.9	10.3	1.3	
South-west Monsoon	June	57.8	41	70.9	0.3	254.9	47.1	1.7	85.2
	July	202.6	97.2	48	35.5	475.7	196.6	0.6	
	August	218.6	101.8	46.6	16	500.1	203.9	0.6	
	September	108.2	71.8	66.4	7.9	289.7	94.3	0.5	
Post-monsoon	October	14.4	18	125.2	0	82.7	6.1	1.7	4.4
	November	7.3	11	151	0	50	3.0	2.4	
	December	8.3	9.6	115.7	0	41.8	5.4	1.8	
Total (Jan. to Dec.)		689.6	187.5	27.5	223.7	1140	674	0.1	

The Post monsoon season is more stable then South-west monsoon. The R^2 value 0.030 means that only 3.0 percent variation in rainfall is explained by time. The highly intensity trends noticed in the month of June to September month get highest rainfall in August month and it reaches its maximum peak and also its start to decreasing from month of October and lowest rainfall in the month of November (Fig. 2).

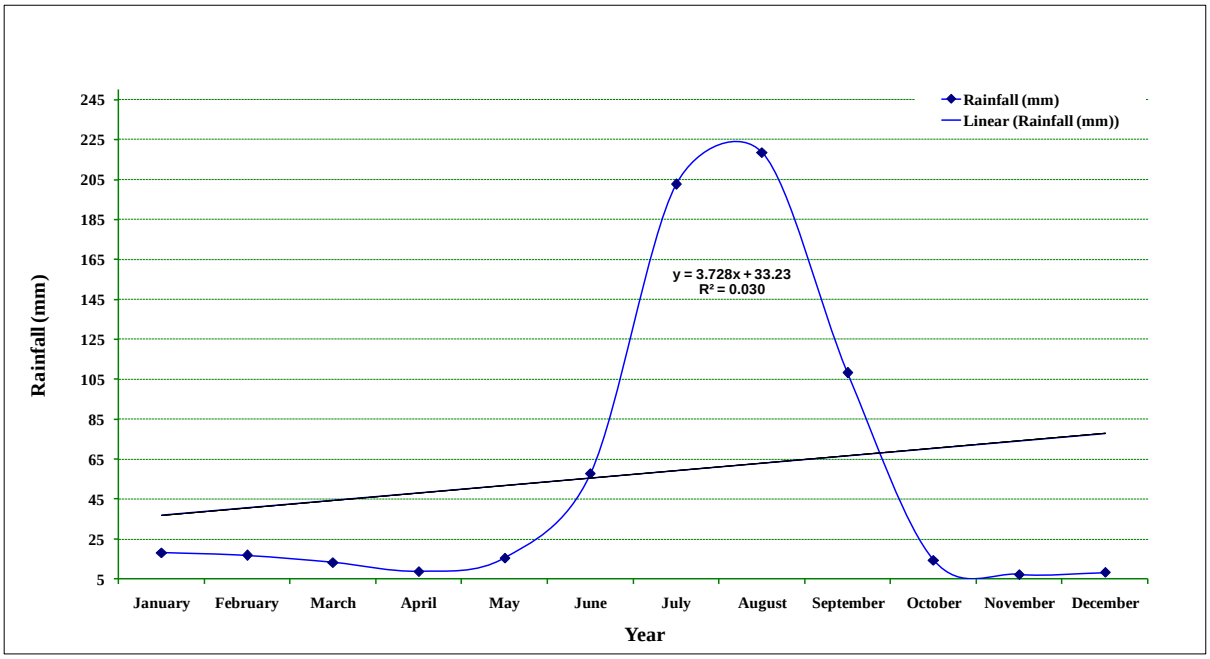

Fig. 2: Average Monthly rainfall of Meerut (1916-2015)

Monthly variation of South west monsoon

The long term data analysis of month wise June, July August and September contributes 10.7%, 33.3%, 38.3% and 17.8% in south west monsoon rainfall season respectively, the results of the analysis are given in Table 5. The mean maximum rainfall is 224.9mm observed in August month whereas minimum rainfall is 62.6mm observed in June month. The minimum rainfall is observed in June , July, August and September month 0.30mm, 36.4mm, 16mm and 9.7mm in year 2012, 2004, 2006 and 1974 respectively, whereas the maximum rainfall is observed 157.8mm, 419.8mm, 500.1mm and 289.7mm in year 2013, 2003, 1995 and 2005 respectively (Table 2 & Fig.3). The monthly south-west monsoon rainfall variability in years is observed maximum after 21th century. It is most important period of rainfall seasonal cycle.

Table 2. Computation of statistical parameters of decadal south-west rainfall data of Meerut district

Monsoon (June-July-Aug-Sept)						Decadal Distribution % of Rainfall
Decade	**Mean (mm)**	**SD (mm)**	**CV (%)**	**Max (mm)**	**Min (mm)**	
1916-1925	587.3	140.9	24	732.4	289.3	100
1926-1935	552.9	204.3	37	1033.3	254.2	94
1936-1945	539	188.5	35	915.2	302.9	92
1946-1955	587.3	150.3	25.6	817.5	363.6	100
1956-1965	669.7	170	25.4	989	486.9	114
1966-1975	661.1	160.9	24.3	958	459.7	113
1976-1985	672.9	172.5	25.6	871.1	326.3	115
1986-1995	607.8	216.6	35.6	896.7	248.1	104
1996-2005	566.8	175.3	30.9	885.8	336.6	97
2006-2015	427	161.5	37.8	696.3	216.1	73
1916-2015	**587.2**	**74.2314**	**12.6419**	**1033.3**	**216.1**	

Fig. 3. Decadal Average South-west rainfall (Monsoon season) of Meerut (1916-2015)

Decadal rainfall pattern

Table 3. Computation of statistical parameters of decadal rainfall data of Meerut district

Decade	Total rainfall decadal					
	Annual Av. Rainfall (mm)	**SD (mm)**	**CV (%)**	**Max. Rainfall (mm)**	**Min. Rainfall (mm)**	**Precipitation ratio (%)**
1916-1925	667	153.9	23.1	845.5	333.1	76.8
1926-1935	648.1	202.9	31.3	1140.3	397.1	114.7
1936-1945	622.9	198.8	31.9	1010	384.1	100.5
1946-1955	671.6	141.3	21	873.8	482	58.3
1956-1965	762.9	169	22.1	1033.2	541.6	64.4
1966-1975	745.9	169	22.7	1035.4	497.3	72.1
1976-1985	786.7	159.8	20.3	956.8	437.4	66
1986-1995	709.6	205.6	29	970.5	355.9	86.6
1996-2005	689.9	191.3	27.7	1056.2	407.7	94
2006-2015	523	206.1	39.4	923.8	223.7	133.9
1916-2015	**682.8**	**76.4**	**11.2**	**1140.3**	**223.7**	**134.2**

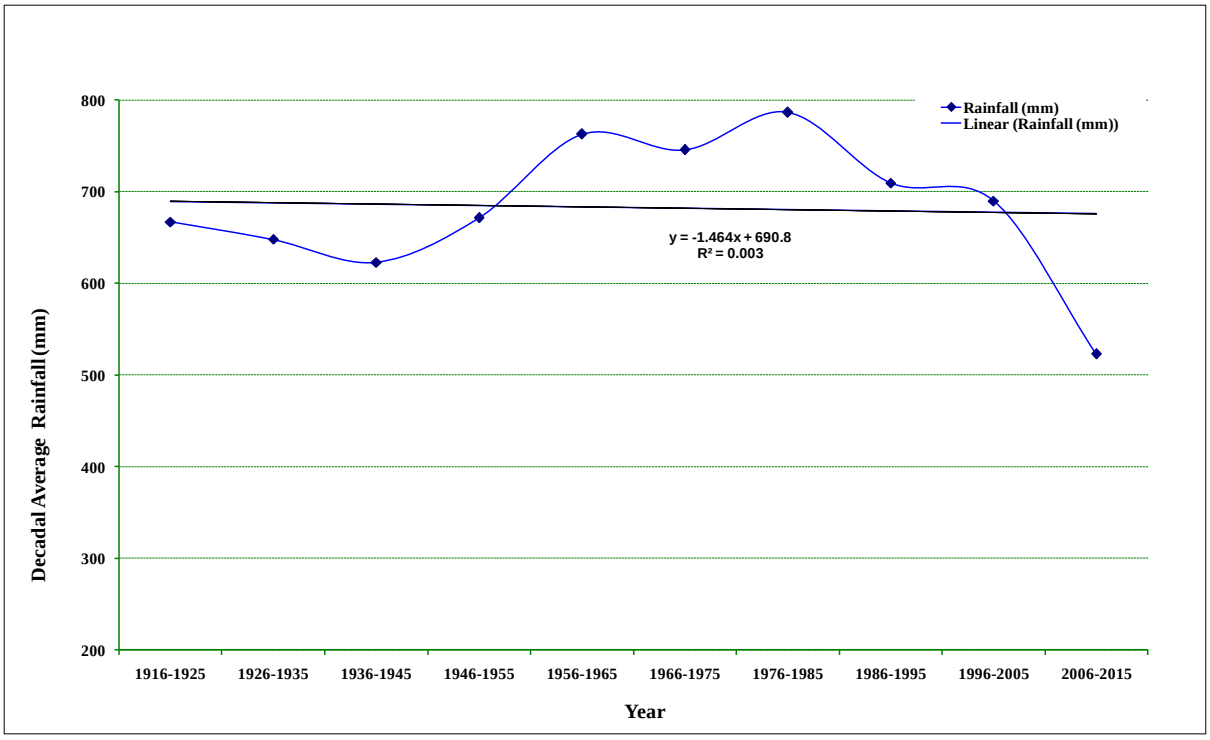

Fig. 4. Decadal Average Annual rainfall of Meerut (1916-2015)

During the 100 year period (1916 to 2015), divide in equal ten decadal rainfall periods for understanding of rainfall data pattern were observed as indicated in Table 3. The analysis of table observed maximum average rainfall 786.7 mm in decadal year 1976-85 and lowest rainfall 523 mm in year 2006-15. The maximum coefficient of variation 39.4% observed in year 2006-2015 whereas minimum coefficient of variation 20.3% observed in decadal year 1976-85. The regression analysis indicates R^2 value of 0.003 for decadal rainfall is observed (Fig.4). The rainfall data variability observed in a decadal period's analysis due to changing of rainfall trend in Meerut.

Precipitation ratio (%)

The abnormalities of rainfall at any location may be brought by a simple ratio of precipitation. It is the difference between maximum and minimum rainfall of the annual rainfall series expressed in terms of mean.

$$P_R = \frac{(P_{Max} - P_{Min})}{P_{Mar}} x100$$

Where,

PR = Precipitation Ratio

P_{Max} = Maximum mean annual rainfall

P_{Min} = Minimum mean annual rainfall

P_{MAR} = Mean annual rainfall

This ratio may give the stability of rainfall with special relationship. Higher the ratio, higher is the abnormality in rainfall and vice versa (Rathod and Aruchamy, 2010). The minimum and maximum precipitation ratios for different decades were worked out for Meerut District is given in Table 3.

In Meerut district, maximum abnormality (i.e. 133.9%) was recorded during 2006-15 (Tables 4) decade during which the district as a whole recorded less annual rainfall. In Meerut, maximum abnormality was recorded during 1946-55 period during which the district recorded very less annual rainfall in precipitation ratio of 58.3%. The overall period (1916-2015) for precipitation ratio was observed 134.2%.

Rainfall departure and cumulative departure of south west rainfall

The departure and cumulative departure from average rainfall for the study area has been depicted in Table.4. The trend of annual departure from the computed value of average annual rainfall reveals that;

(a) Years showing annual positive departure with respect to average annual rainfall were 1916-17, 1921-24, 1926, 1933-34, 1936, 1942, 1944-45, 1948-1950, 1953, 1955, 1957-58, 1960-61, 1963-1964, 1966-67, 1969, 1971-72, 1975-1978, 1980, 1982-83, 1985, 1988, 1990, 1993-96, 1998, 2000, 2003-04, 2010 & 2013. The positive trend of rainfall shows the favourable conditions for recharge.

(b) Years showing annual negative departure with respect to average annual rainfall were 1918-1920,1925, 1927-32, 1935, 1937-1941, 1943, 1946-47, 1951-52, 1954, 1956, 1959, 1962, 1965,1968,1970,1973-74,1979,1981,1984,1986-87,1989,1991-92,1997, 1999, 2001-04, 2005-09, 2011-12 & 2014-15. The negative trend of rainfall shows the unfavourable conditions for recharge.

Table 4. South –west rainfall data and its departure and cumulative departure from average South-west rainfall in Meerut district (1916-2015)

South-west rainfall (mm) (June-July-August-September)									
1916-2015									
Year	**Standardized rainfall anomaly**	**Departure from average rainfall**	**Cumulative departure from average rainfall**	**Season Trend**	**Year**	**Standardized rainfall anomaly**	**Departure from average rainfall**	**Cumulative departure from average rainfall**	**Season Trend**
1916	0.77	96.8	-96.8	605.3	**1966**	0.14	24.8	-582.4	587
1917	0.8	162.7	65.9	604.9	**1967**	1.35	260.8	-321.6	586.6
1918	-1.64	-349.7	-283.8	604.5	**1968**	-0.32	-97.6	-419.2	586.3
1919	-0.82	-146.1	-429.9	604.2	**1969**	0.39	33.2	-386	585.9
1920	-0.46	-124.2	-554.1	603.8	**1970**	-0.51	-36.3	-422.3	585.5
1921	0.35	21.3	-532.8	603.4	**1971**	1.18	230.2	-192.1	585.2
1922	0.41	39.3	-493.5	603.1	**1972**	0.53	64.9	-127.2	584.8
1923	-0.02	22	-471.5	602.7	**1973**	-0.05	-15.7	-142.9	584.4
1924	0.56	134.5	-337	602.3	**1974**	-0.7	-185.5	-328.4	584.1
1925	0.05	-14.5	-351.5	602	**1975**	2.04	352.7	24.3	583.7
1926	0.16	23.9	-327.6	601.6	**1976**	1.54	274	298.3	583.3
1927	-0.58	-78.6	-406.2	601.3	**1977**	0.48	117.8	416.1	583
1928	-1.83	-285.6	-691.9	600.9	**1978**	1.56	246.7	662.8	582.6
1929	-1.17	-233.9	-925.8	600.5	**1979**	-1.44	-245.3	417.4	582.3
1930	-0.37	-65.9	-991.6	600.2	**1980**	1.28	201.9	619.4	581.9
1931	-0.05	-6.7	-998.3	599.8	**1981**	-0.35	-27.4	592	581.5
1932	-0.48	-142.6	-1140.9	599.4	**1982**	-0.08	89.2	681.2	581.2
1933	2.46	457.5	-683.4	599.1	**1983**	0.44	179	860.2	580.8
1934	0.35	44.6	-638.8	598.7	**1984**	0.17	-11.8	848.4	580.4
1935	-0.38	-59.5	-698.3	598.3	**1985**	1.11	215.2	1063.5	580.1
1936	1.81	327.3	-371	598	**1986**	-0.64	-85.9	977.7	579.7
1937	-0.62	-110.1	-481.1	597.6	**1987**	-1.87	-326.9	650.8	579.3
1938	-1.15	-228.6	-709.8	597.2	**1988**	1.7	287.7	938.5	579
1939	-1.05	-236.7	-946.5	596.9	**1989**	-1.08	-179.4	759.1	578.6
1940	-0.7	-132.1	-1078.6	596.5	**1990**	1	207.7	966.8	578.2
1941	-1.56	-298.6	-1377.2	596.1	**1991**	-0.25	-24.8	942	577.9
1942	0.76	117.2	-1260	595.8	**1992**	-0.8	-128.6	813.4	577.5
1943	-0.68	-181.8	-1441.8	595.4	**1993**	1.13	161.4	974.8	577.1
1944	-0.01	79.1	-1362.6	595	**1994**	0.76	114.6	1089.4	576.8
1945	0.57	65.7	-1296.9	594.7	**1995**	1.18	242.9	1332.3	576.4
1946	-0.17	-68.1	-1365	594.3	**1996**	1.13	184.7	1517	576
1947	-0.56	-132	-1497	593.9	**1997**	-0.58	-35.6	1481.4	575.7
1948	0.92	173.2	-1323.8	593.6	**1998**	0.58	136.6	1618	575.3
1949	0.74	89.4	-1234.4	593.2	**1999**	-1.38	-275	1342.9	574.9
1950	1.27	191	-1043.4	592.8	**2000**	-0.07	8.2	1351.1	574.6
1951	-1.23	-200.8	-1244.2	592.5	**2001**	-0.62	-99.4	1251.8	574.2
1952	-0.82	-156.4	-1400.6	592.1	**2002**	-1	-217.8	1034	573.8
1953	0.26	17.4	-1383.1	591.8	**2003**	1.64	373.4	1407.4	573.5
1954	-0.7	-110.1	-1493.3	591.4	**2004**	-0.75	39.5	1447	573.1
1955	0.3	84.4	-1408.9	591	**2005**	-0.08	-43.4	1403.6	572.8
1956	-0.2	-10.8	-1419.7	590.7	**2006**	-1.73	-330.1	1073.5	572.4
1957	0.11	70.5	-1349.2	590.3	**2007**	-1.64	-309.1	764.5	572
1958	1.53	258.5	-1090.7	589.9	**2008**	-1.13	-245.4	519.1	571.7
1959	-0.3	-62.4	-1153.1	589.6	**2009**	-2.04	-459.1	60	571.3
1960	-0.01	5.5	-1147.6	589.2	**2010**	0.41	19.1	79.2	570.9
1961	1.44	314	-833.6	588.8	**2011**	-0.49	-126.6	-47.4	570.6

1962	-0.11	-35.9	-869.4	588.5	**2012**	-1.43	-287.4	-334.8	570.2
1963	0.43	52.9	-816.5	588.1	**2013**	0.6	241	-93.7	569.8
1964	2.21	350.5	-466	587.7	**2014**	-0.75	-48.1	-141.8	569.5
1965	-0.55	-141.1	-607.2	587.4	**2015**	-0.62	-51.8	-193.6	569.1

Standardized rainfall anomaly

Table 4 depicts the computed annual mean rainfall , departure & cumulative departure of rainfall , seasonal trend and standardized anomalies within the year under consideration (1915-2016) over Meerut District. Fig. 5. shows the standardized rainfall deviations *viz.* 1916-17, 1921-22, 1924-26, 1933-34, 1936, 1942, 1945, 1948-50, 1953, 1955, 1957-58, 1961, 1963-64, 1966-67, 1969, 1971-72, 1975-78, 1980, 1983-85, 1988, 1990, 1993-96, 1998, 2003, 2010 & 2013 are years with above average rainfall with 1933 showing the highest positive rainfall anomaly while the other years show rainfall below normal with 2009 showing the lowest negative rainfall deviation.

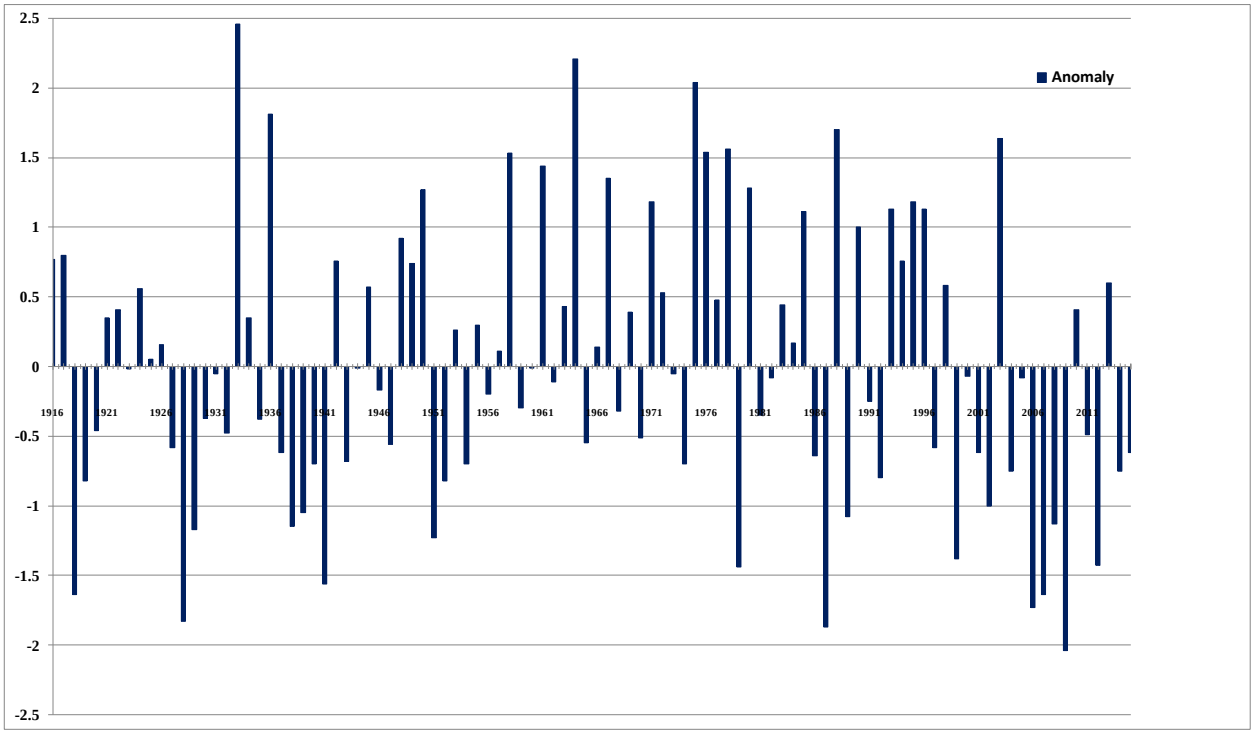

Fig. 5. Standardized rainfall anomaly over Meerut District from 1916-2015

Trend analysis of south-west rainfall (Monsoon season)

A trend analysis of the average south west rainfall of Meerut district for 100 year period from 1916 to 2015 was statistical test MS Excel in Fig. 6 shows the plots of annual and south-west rainfall for the study area; trends lines for the data have also been drawn. The monsoon season (S-W) is least scatter then annual rainfall. It does not show much scatter. It gives valuable trend information of series observations. Trend analysis was also performed on seasonal scale to examine if there are trends in the data at this scale. The trend analysis helps to measure the deviation from the trend and also provides information pertaining to the nature of trend. The analysis can be used as a tool to forecast the future behaviour of the trend. The method of least square fit for straight line has been used for trend analysis of the behaviour of annual rainfall, south west rainfall and rice yield. After trend analysis of data observed rainfall trend is going to decline pattern.

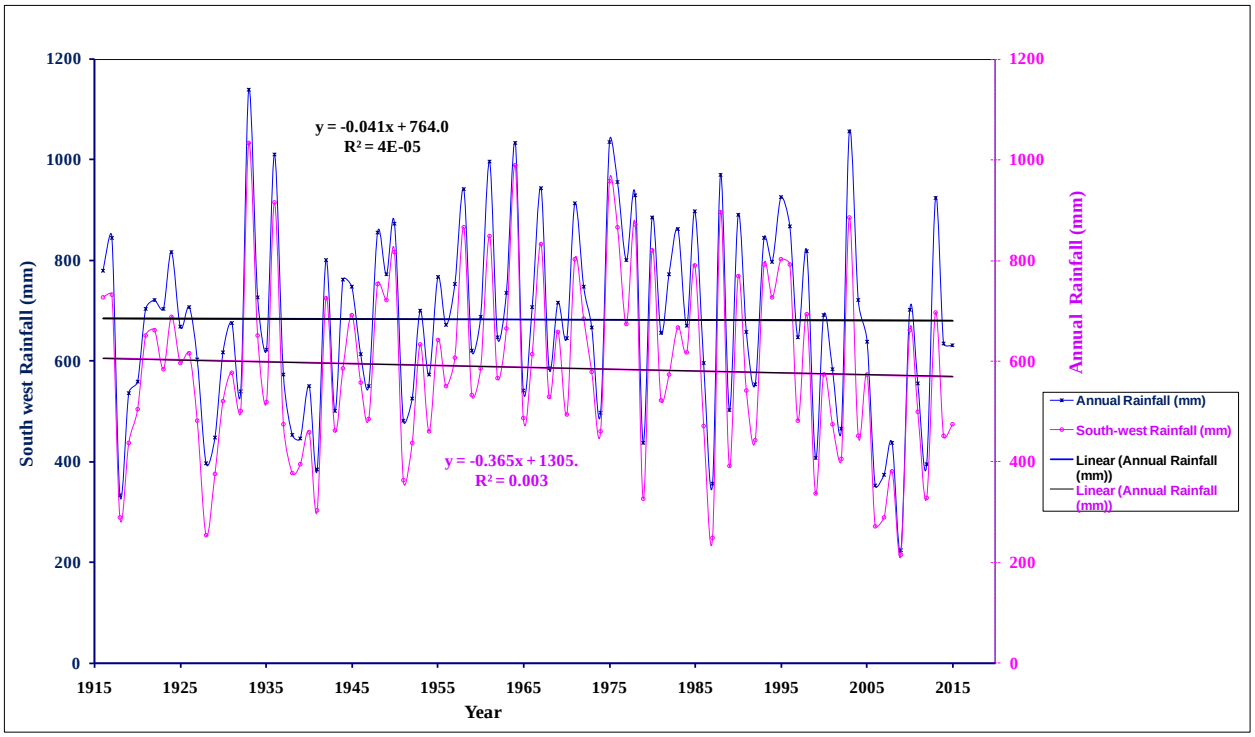

Fig. 6. Trend of rainfall in Meerut district (1916-2015)

Monsoon Precipitation Index (MPI)

Moetasim Ashfaq *et al.* (2009) defined the precipitation index as the departure of rainfall from the climatological means (1961–1990), averaged over land points between 70º - 90ºE and 5º–25ºN. Calculation of MPI is useful for both agricultural and hydrological applications. Since MPI is not adversely affected by the topography it gives us an idea about spatial variation of monsoon rainfall over different topographical regions. Higher the MPI, lesser is the rainfall variation at individual district. In the present study on the basis of available monthly rainfall data, monsoon precipitation index (MPI) has been calculated as,

Monsoon Precipitation Index

$$(\text{MPI} = \frac{\text{Annual Range}}{\text{Total Annual Rainfall}}$$

Where, Annual Range = (Monsoon rainfall – Non-monsoon rainfall)

MPI in case of Meerut mostly varied from 0.71 to 0.76 except in the decadal year 1996-2005 & 2006-05 it was 0.64 and 0.63 respectively (Table 5). The lowest MPI of 0.64 to 0.63 was recorded due to district received less rainfall during the monsoon months.

Table 5. Decadal Monsoon Precipitation Index (MPI) of Meerut

Sl. No.	Decade	MPI VALUE
1	1916-1925	0.76
2	1926-1935	0.71
3	1936-1945	0.73
4	1946-1955	0.75
5	1956-1965	0.76
6	1966-1975	0.77
7	1976-1985	0.71
8	1986-1995	0.71
9	1996-2005	0.64
10	2006-2015	0.63
1916-2015		**0.72**

Forecasting of annual rainfall and S-W rainfall

On the basis, the future forecast of rainfall and yield amount for a period of ten years from 2016 to 2026 has been made (Table 6), which shows a negative trend for the coming years. In future, expected annual and south west rainfall may be less in year

2026 observed 680.3mm and 565.1mm in Meerut district. The expected annual rainfall in year 2016 to 2026 rainfall patterns are declining stage. The trend analysis gives the scenario of current to expected future situation. Monsoon rainfall is one of the key factor play vital role in Indian agriculture. Our statistical result indicates that monsoon rainfall is affecting the kharif season in the study area.

Table 6. Expected future Annual & South-west rainfall (mm) of Meerut District

Year	Expected future rainfall trend (mm)	
	Annual	**South-west Monsoon**
2016	680.7	568.7
2017	680.6	568.4
2018	680.6	568.0
2019	680.6	567.6
2020	680.5	567.3
2021	680.5	566.9
2022	680.4	566.5
2023	680.4	566.2
2024	680.3	565.8
2025	680.3	565.4
2026	680.3	565.1

CONCLUSION

Water is a vital component and rainfall is major source of irrigation especially for south west monsoon for Kharif. Analysis of rainfall data of a century (1916-2015) over Meerut plays a significant role in the agricultural and urbanization contribution and in the overall growth of the District. The impacts of climate changes on temporal and spatial patterns are clearly noticed in this analysis. In the century based rainfall data analysis observe in maximum rainfall 115% in decadal period year 1976-85 whereas in decadal period year 2006 to 2015 only 73% monsoon rainfall was observed.

The data of annual rainfall and S-W rainfall is 689.6mm and 587.2mm respectively. The monthly south-west monsoon rainfall variability in years is observed maximum after 21th century. It is most important period of rainfall seasonal cycle. The analysis data of S-W monsoon observed highest rainfall 85.2% and lowest rainfall in post monsoon season 4.4%. The analysis of trend of rainfall and observed rainfall is declining. The District experienced irregular pattern rainfall which adversely affected the agriculture production and yield. There is increase in annual and South-west Monsoon season CV for all the decades during 1915-2016 periods ranging between 27.5 for annual and 12.6 for monsoon rainfall.

Statistical indicators viz. coefficient of skewness showed that the frequency of low precipitation is high in Meerut during winter and pre monsoon more or less equal but south west rainfall more than other season and average post-monsoon season has very low rainfall.

The anomalous departures from the mean were observed the highest positive and negative departure from the mean of approximately -459.1 & 457.5 in year 2009 and 1933 respectively.

The analysis included variability of rainfall, trends in rainfall pattern and changes in spatial and temporal patterns of Precipitation Ratio and Monsoon Precipitation Index. Due to plain topography, the annual and seasonal rainfall in the district has been estimated by a simple ratio of precipitation. The maximum abnormality 2.46 and -2.04 in annual rainfall was recorded during 1933 & 2009. It is seen that the average MPI varied from 0.63 to 0.77.

The trend in the annual rainfall showed that the rainfall decreasing in the area whereas south-west rainfall declined pattern of 3% changes was also observed in the century.

The standardized anomalies results obtained show a fluctuating rainfall pattern across the years over Meerut District which makes it hard to freely forecast rainfall trend for a future season. The information is useful for agriculturists and policy makers on critical issues is it affects seasonal

agricultural practices such application of agricultural inputs, water resources maintenance and management practices. The rainfall data analysis of Meerut District for a period of 100 years (1916 to 2015) reveals variation in the rainfall amount and points out a negative trend of rainfall in future. The global climate and the local environmental changes are the chief factors for the variation in rainfall over the recent times. The knowledge of current situation of weather and climate change related pattern and adaptation of technology is maintend trend. Uncertainty on the dates of monsoon onset and its withdrawal also puts a great problem before the farmers.

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b.Rainfall year 2003: http://shodhganga.inflibnet.ac.in/bitstream/10603/41261/6/06_chapter%202.pdf

c.Rainfall data of Meerut (2004 to 06): http://www.indiawaterportal.org/met_data/

d. IMD (Indian meteorology Department, New Delhi (2007 to2013): Hydromet Division, India Meteorological Department, District rainfall (mm) for last five years (2007 to 2013)

e. NASA/POWER Agroclimatology Daily Averaged Data: (2014-15): http://power.larc.nasa.gov/common/AgroclimatologyMethodology/Agro1d0_Methodology_Content.html & http://power.larc.nasa.gov

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RESEARCH ARTICLE

ASSESSMENT OF GENETIC FIDELITY OF MOTHER PLANT AND *IN VITRO* RAISED MEDICINAL PLANT *EPHEDRA GERARDIANA* THROUGH MOLECULAR MARKERS

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Abstract: *Ephedra gerardiana* is an important medicinal gymnosperm shrub. It has been traditionally use for an assortment of medicinal purpose. Molecular markers analysis was conducted to screen genetic fidelity among *in vitro* raised plantlets compare with mother plant of *Ephedra gerardiana*. Genetic fidelity of regenerated plants was assessed using Random Amplified Polymorphic DNA (RAPD) and Simple Sequence Repeat (SSR) Primers. A total of 50 RAPD primers and 30 SSR primers were utilized in the present study to analyze genetic fidelity of mother plant and among tissue culture raised plants of *Ephedra gerardiana*. Out of 50 RAPD primers, 19 primers exhibited DNA amplification in all the DNA samples and out of 30 SSR primers, 18 were show amplification. The amplified products of the regenerated plants showed similar banding patterns to that of the mother plant thus demonstrated the homogeneity of the micropropagated plants. The banding pattern ruled out presence of any kind of somaclonal variation. Thus, the results revealed that genetic fidelity between the micropropagated and mother plant in *Ephedra gerardiana* and supports the suitability of tissue culture technique for generation of genetically similar plants. Hence, the results obtained confirmed genetic stability of regenerated plants.

Keywords: *Ephedra gerardiana*, Micropropagation, Genetic fidelity, RAPD, SSR

INTRODUCTION

Ephedra is a gymnospermic genus of Gnetales and family Ephedraceae. Ephedra belongs to class Genetopsida having 3 different genera Ephedrales, Gnetales and Welwitschiales respectively (sharma *et al.,* 2012). *Ephedra*, also known as ma huang, contains the ephedrine alkaloid that stimulates the central nervous system (Tod and Stewart 1997). *Ephedra* have significant medicinal properties due to the presence of various keenly secondary metabolites. Ephedrine alkaloids have an adrenaline like effect on the body it excites the nervous system, opens blood vessels, and stimulates the heart (www.ephedrafacts.com). The *Ephedra sinica* plant contains ephedrine alkaloids that are used in the herbal supplements once they have been cultivated from the dried stems of the plant. Some of the main alkaloids in *Ephedra* are ephedrine and pseudoephedrine, which can be found in over the counter (OTC) drugs, but an important distinction is that the alkaloids in OTC drugs are produced synthetically (Paul 2001). Synthetic ephedrine is more potent than the ephedrine alkaloids found in ephedra used mainly as a bronchial decongestant, ephedrine is found in bronchodialators such as Primatene, while pseudoephedrine is commonly utilized in decongestants such as Sudafed. Physiologically, it acts to expand breathing passages, constrict blood vessels, and increase arterial blood pressure. It is the increase in arterial blood pressure that causes severe hypertension, stroke, or heart attack by Jody (1997). Plant tissue culture appears to be a reliable method for the production of *Ephedra* within a short time span. Among the various methods of *in vitro* propagation, shoot proliferation is considered to be the least susceptible to genetic modification (Shenoy *et al.,* 1992). Somaclonal variation has been reported to some extent at different levels i.e. morphological, cytological, cytochemical and molecular in micropropagated plants (Rani and Raina 2000). The genetic analysis of micropropagated plants using a multidisciplinary approach, especially at the DNA sequence level initially and at various cultural stages, it is essential to maintain genetic integrity of micropropagated plants. Several cytological, isozyme and molecular markers have been used to detect the variation and/or confirm the genetic fidelity in micropropagated plants (Gupta *et al.,* 2009; Kumar *et al.,* 2011). The sensitivity, reproducibility and strong discriminatory power of microsatellite and RAPD markers (Parida *et al.,* 2009; Sharma *et al.,* 2013) make them suitable for detecting somaclonal variation. However, their application in the study of somaclonal variation has been quite limited.

In the present study, *Ephedra gerardiana* were propagated using shoot buds as the explants. The

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micropropagated plants were successfully transferred to the field. The genetic fidelity of *in vitro* raised plants was assayed using RAPD and SSR markers to assess the genetic stability among the regenerated plantlets.

MATERIAL AND METHOD

Plant collection

Ephedra gerardiana were collected from Natural habitat of Chakrata forest division in the Uttarakhand state and were grown in the polyhouse of the K.L.D.A.V (PG) College Roorkee.

In vitro culture of *Ephedra gerardiana*

In vitro culture of *Ephedra gerardiana* was followed by sharma *et al* 2012. Regenerated plants genomic DNA were used for genetic fidelity analysis with mother plant.

Genomic DNA extraction

Stem tissue was used for DNA extraction of mother plant as well as regenerated plantlets. Initially cut stem tissue into small pieces and put into liquid nitrogen after that grind using mortar and pestle. DNA extraction was made using modified CTAB procedure (Doyle and Doyle 1990). The quantity of isolated DNA was determined by using Biophotometer (Eppendorf, Germany), visualized on 0.8% agarose gel stained with ethidium bromide and a final concentration of 80ng was used for PCR.

Development of SSR and RAPD primer

ESTs sequences of *Ephedra* were retrieved from available data base NCBI (http://www.ncbi.nlm.nih.gov/) and were screened for presence of microsatellites using CAP 3 and MISA tools (Thiel *et al*., 2003, downloaded from http://pgrc.ipk-gatersleben.de/misa/), Finally SSR primers were designed using Primer 3 software.

RAPD and SSR PCR amplification

The obtained DNA was diluted as required working concentration for PCR analysis using RAPD and SSR primers. Both primers amplification was performed using a thermal cycler (Veriti, ABI, Germany).

For RAPD analysis the reaction mixture composed of 100 ng DNA, 1X Taq buffer, varying the $MgCl_2$ concentration 1.5 mM to 3.0 mM, 10 pmoles primer, 0.2 mM dNTPs and 1 U of Taq DNA polymerase (Genei, Bangalore, India). The amplification program was as follows; initial denaturation at 95°C for 5 min followed by 45 cycles of denaturation at 94°C for 40s, annealing at 34-40°C for 1.5 min, extension at 72°C for 1 min and finally ending with one cycle at 72°C for 10 min. After amplification, PCR products were stored at −20°C till performing electrophoresis. Amplified products were run in 1.5% agarose gel and submerged in 1x Tris-Acetic acid- EDTA (TAE) buffer (pH 8.0) at a constant voltage (60 V).

For the SSR analysis the reaction mixture composed of 80 ng DNA, 1X Taq buffer, 1.5 mM $MgCl_2$, 10 pmoles each of forward and reverse primers, 0.2 mM dNTPs and 1 U of Taq DNA polymerase (Genei, Bangalore, India). The amplification program was as follows; initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 94°C for 30s, annealing at 50-58°C for 45s, extension at 72°C for 1 min and finally ending with one cycle at 72°C for 7 min. After amplification, PCR products were stored at −20°C till performing electrophoresis. Amplified products were run in 2.5% agarose gel and submerged in 1x Tris-Acetic acid- EDTA (TAE) buffer (pH 8.0) at a constant voltage (60 V). The profiles were documented by UV trans-illuminator equipped gel documentation system (Avegene, Taiwan). The molecular size of amplified fragments was compared with 100 bp DNA ladder (Genei, Bangalore, India).

Data analysis

The scorable bands were included in the analysis. Each band was considered a single locus and 1-0 matrices were formed for further analysis. Presence and absence of each locus was coded by 1 or 0 respectively. The binary data were used to compute pairwise similarity coefficients and the similarity matrix thus obtained was subjected to cluster analysis using UPGMA (unweighted par group method with arithmetic average) using NTSYS-PC version 2.01 program for cluster analysis (Nei, 1971).

RESULT AND DISCUSSION

Development of ESTs SSR markers from available data

Screening of 4,982 ESTs related to *Ephedra* to identification of 931 microsatellite sites. In the current analysis, mononucleotide repeats were not included because of the abundance of poly A/T repeats, arising due to the presence of Poly A/T tails in cDNA molecules and errors prevailing due to sequencing (Jain *et al.*, 2010; Jain *et al.*, 2014). In total 3.71% of the ESTs were found to carry microsatellites within them which are quite comparable to some of the previous studies conducted on other plants (Victoria *et al.*, 2011). The tri-nucleotide repeat motifs were the most prevalent (54.13%) class of microsatellites quickly followed by di-nucleotide (36.53%) and tetra-, penta- and hexa-nucleotide repeats together contributing 9.34% (Figure 1). However results observed in the current study contradicts the tri-nucleotide abundance observed by Parida *et al.* (2010). One possible reason behind this observation could be the small data set considered in the present study. Considering the length of microsatellite loci, 64.9 % microsatellites were grouped as class II microsatellite repeats (< 20 nucleotide long) and 35.1 % microsatellites as class I microsatellite repeats (≥ 20 nucleotide long). Similar preponderance of class II repeats has been reported in other studies also (Roorkiwal and Sharma, 2011; Jain *et al.*, 2014). The

inverse relationship between the length of microsatellites and their abundance holds true in the present study. This could be due to the fact that longer repeats have downward mutation bias (Katti *et al.*, 2001).

The total classified frequency numbers of dinucleotide repeats were observed to be 50.36% followed by AG repeats, 30.66% AT repeats and AC repeats 18.98% (Figure 2). Among the dinucleotide repeats, the most and least frequent motifs were GA and AT respectively. Similar abundance of GA dinucleotide repeats, has been observed in many other plant species in the recent past (Sharma, 2011; Victoria *et al.*, 2011). This repeat class is known to play an active role in gene regulation in both animal and plant species (Trivedi, 2004). In trinucleotide repeats, AAG repeat class was found to be the most prevalent trinucleotide repeat followed by ATC repeat class (Figure 3,4), which could be possibly due to their high AT content (Cordeiro *et al.*, 2003; Pinto *et al.*, 2004) and consequent usage bias in the coding sequences (Morgante *et al.*, 2002).

The assessment of the genetic stability of *in vitro* plant is vital step in the study of tissue culture technique for regeneration of genetically true-to-type plantlets (Katti *et al.*, 2001). Although the present scenario plant conservation is predominantly depends on micropropagation methods. However, genetic and phenotypic variants are reported during in this method, which may be the focal elucidation to originating somaclonal variants. Thus, the risks of genetic change induced by micropropagation and the important of assessing the genetic stability must be considered in the perspective of conservation.

The present study was conducted to screen genetic fidelity in *Ephedra gerardiana* regenerated plantlets through direct organogenesis using Nodal segment as explant. PCR based RAPD and SSR technique has been frequently used for genetic fidelity in this medicinal shrub plants.

In the present investigation 50 RAPD primers were used, amongst which only 19 primers show banding pattern (Figure 5). The number of scorable band for each primer varied from 6-15 with an average of 10.68 bands present per locus (Table 1.1, 1.2) and found 96% genetic stability with mother plant (Table 1.3 , Figure 7).

Out of 30 SSR primers 18 SSR primers show the scorable band pattern (Figure 6). Total 33 amplicon were amplified with average 1.83 per locus. That show low polymorphism on an average 0.09 and maintain more than 99 % genetic similarity with mother plants (Table 1.4, 1.5, 1.6, Figure 8). Markers results illustrate the high genetic similarity of tissue culture plant with mother plant. It is the sign of no somaclonal variation at genetic level. Similarity matrix based on Jaccard's coefficient revealed that pairwise value between the mother plant and its tissue cultured plants ranged from 0.86 to 0.95 with RAPD primers and among tissue cultured plants, it was 0.96 to 1.00 with SSR primers, thus indicating a high degree of genetic fidelity. Many factors are responsible for inducing variability during micropropagation such as explants source, time of culture, number of subcultures, phytoharmone, genotype, media composition and the level of ploidy.

Saini *et al*. (2004) have reported that RAPD analysis of sugarcane showed 90% of genetic purity among its micropropagated plants. Further, Jain *et al*. (2005) also found no significant variation among meristem derived RAPD analysis. Independent studies conducted by Devarumath *et al*. (2007), Lal *et al*. (2008) and Tawar *et al*. (2008) on plants propagated from apical meristems reported high rate of genetic fidelity. RAPD marker banding patterns have been the source behind these results. They found more than 97% genetic similarity between mother plant and micropropagated plant and concluded these tissue cultured plants were genetically stable. Sharma *et al*. (2007) evaluated genetic fidelity of micropropagated plantlets of medicinal plant using RAPD markers. They observed no genetic variation and no somaclonal variation between mother plant and micropropagated plantlets. Besides them, Suprasanna *et al*. (2006, 2007), Lal *et al*. (2008) and Pandey *et al*. (2012) in their respective studies also analyzed genetic fidelity in *Saccharum officinarum* regenerated plantlets through RAPD and SSR markers. Results obtained from banding pattern obtained from both the makers indicated no sign of somaclonal variation and confirmed genetic stability of regenerated plants.

SSRs are sensitive, reproducible and have strong discriminatory power (Parida *et al*., 2009) and thus, can be used to detect the genetic variations and somaclonal variation but their use in detecting the clonal fidelity is quite restricted. Though there are few reports of testing genetic fidelity in many plants using various molecular markers (Taylor *et al*., 1995; Saini *et al.*, 2004; Suprasanna *et al.*, 2006; Devarumath *et al*., 2007; Lal *et al.*, 2008), 90-100% genetic fidelity using SSR marker has been reported in medicinal plants such as *Centurea ultreiae*, (Pandey *et al.*, 2012).

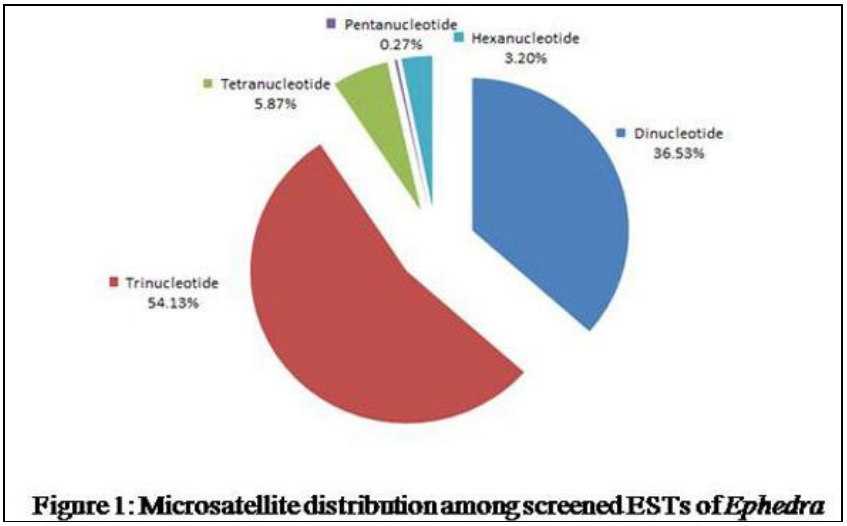

Figure 1: Microsatellite distribution among screened ESTs of *Ephedra*

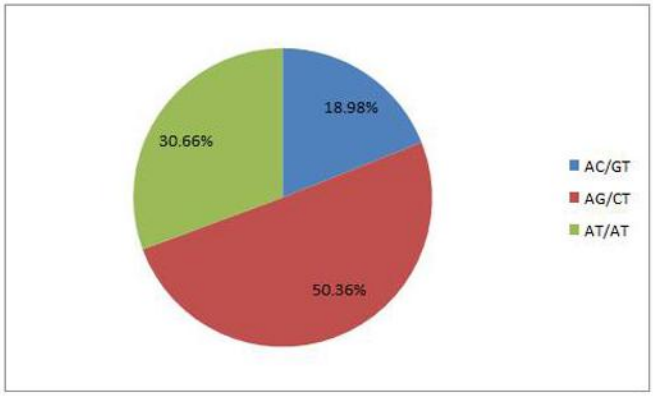

Figure 2: Individual motif abundance among di-nucleotide repeats in *Ephedra* ESTs

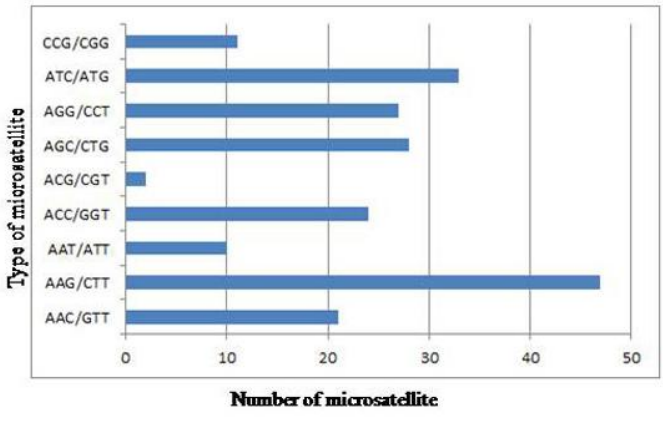

Figure 3: Individual motif abundance among Tri-nucleotide repeats in *Ephedra* ESTs

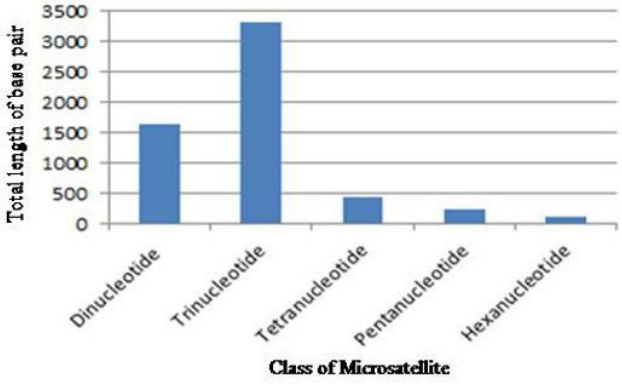

Figure 4: Length contribution by each class of Microsatellite

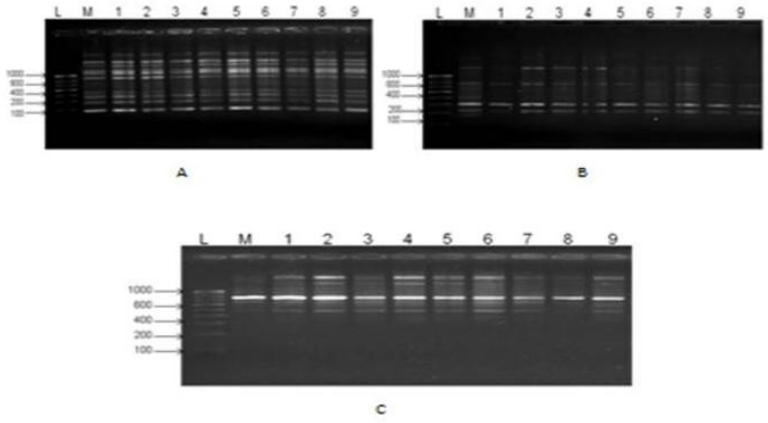

Figure 5: A,B,C RAPD primer number 2,14,16 (Table) on 1.5 % Agarose gel Lane M - Mother Plant; Lane 1 to 9 regenerated plantlets of *Ephedra gerardiana*; Lane L - 100 bp ladder.

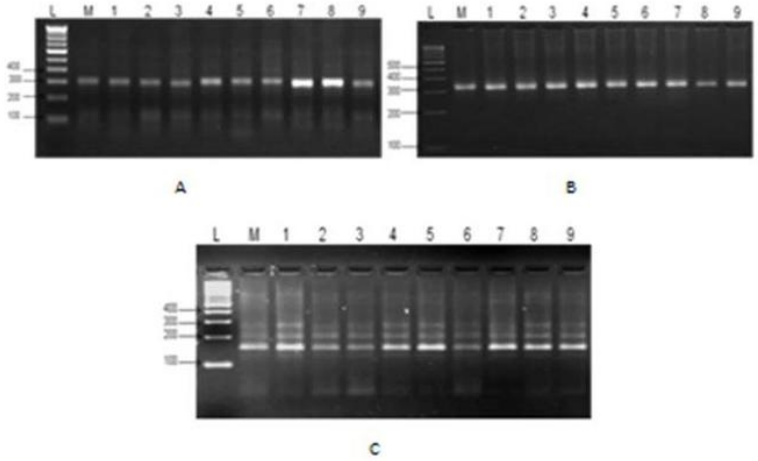

Figure 6: A,B,C SSR primer pair number 1,4,7 (Table) on 3% metaphor gel Lane M - Mother Plant; Lane 1 to 9 regenerated plantlets of *Ephedra gerardiana*; Lane L - 100 bp ladder.

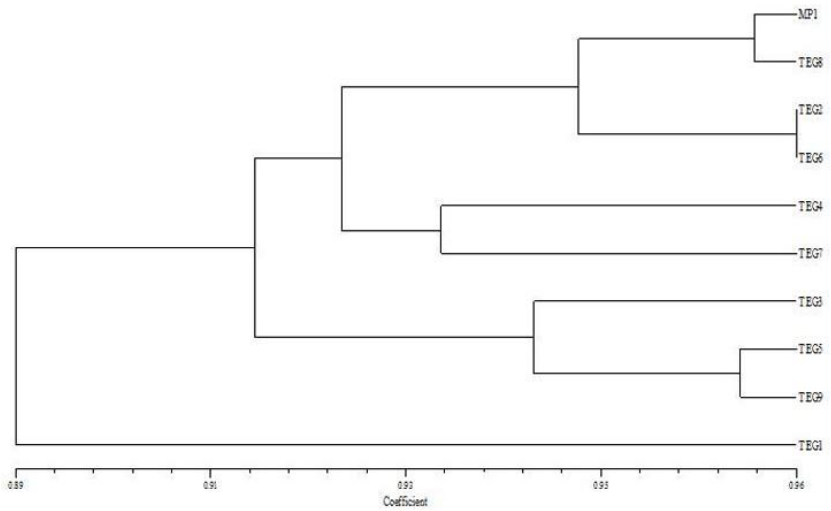

Figure 7: Dendrogram show the genetic similarity of the mother plant with tissue culture plantlets on the basis of jacard coefficient using RAPD primers

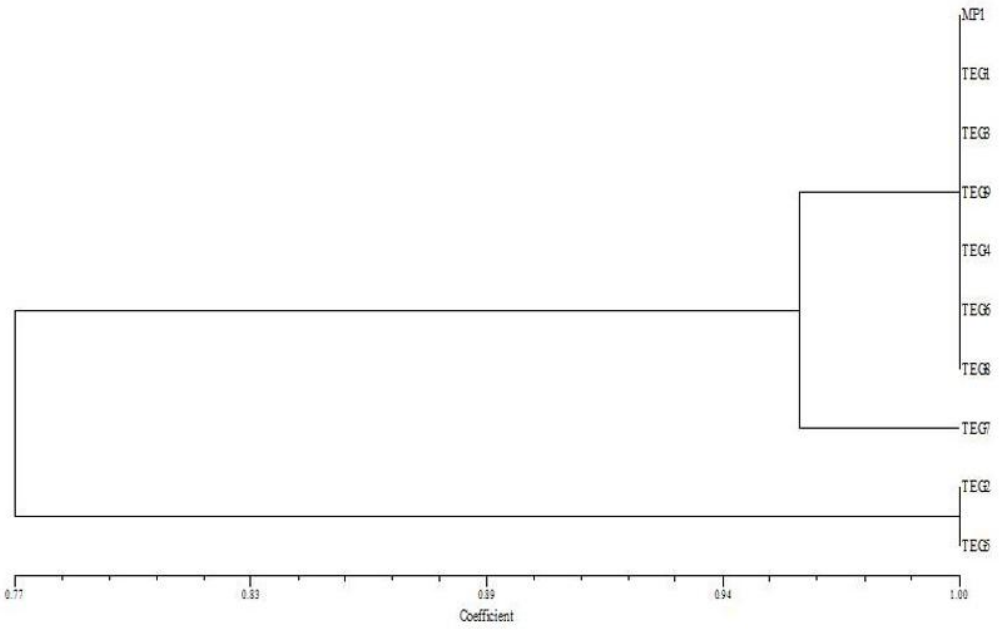

Figure 8: Dendrogram show the genetic similarity of the mother plant with tissue culture plantlets on the basis of jacard coefficient using SSR primers

CONCLUSION

From the study, it was concluded that the genetic stability of *Ephedra gerardiana* plantlets observed in our study indicates good genetic fidelity, when examined by two marker systems. A polymorphic rate was detected very low due to the point mutations or any changes occurs the primer binding side. It was also found more than 90% plantlets similar with the mother plant. Molecular marker is beneficial for the studies of phylogenetic analysis, cluster analysis and genetic fidelity of the plant.

Table 1. List of primers exhibiting DNA amplification in RAPD analysis of *Ephedra gerardiana*

S. No.	Primers	Sequence of primers -5'3'	Amplified Band	Polymorphic Band
1	OPP-01	**5'**-GATGCACTCC	8	1
2	OPP-02	**5'**-TCGGCACGCA	10	2
3	OPP-05	**5'**-CTGTATCGCC	13	3
4	OPP-07	**5'**-GTGTCTCAGG	7	1
5	OPP-11	**5'**-GTGGGCTGAC	15	2
6	OPP-12	**5'**-GTCCATGCCA	11	2
7	OEP-03	**5'**-CCAGTAGCAC	10	4
8	OEP-07	**5'**-AGATGCAGCC	9	2
9	OEP-11	**5'**-GAGTCTCAGG	13	5
10	OEP-12	**5'**-TTATCGCCCC	11	1

11	OEP-14	**5'**-TGCGGCTGAG	14	2
12	OEP-16	**5'**-GGTGACTGTG	15	4
13	OEP-17	**5'**-CTACTGCCGT	12	1
14	OPW-02	**5'**-ACCCGGCCAA	6	0
15	OPW-03	**5'**-GTCCGGAGTG	8	2
16	OPW-04	**5'**-CAGAAGCGGA	7	0
17	OPW-05	**5'**-GGCGGATAAG	9	2
18	OPW-06	**5'**-AGGCCCGATG	12	4
19	OPW-07	**5'**-CTGGACGTCA	13	3
Total No. of bands			**203**	**41**

Table 2. Data of RAPD analysis

S. No.	Parameters	Readings
1	Number of assay	19
2	Number of amplicon	203
3	Average Number of amplicon	10.68
4	Average number of polymorphic amplicon	2.16
5	Percentage polymorphism	21.6

Table 3. Nei's genetic similarity Jacard coefficient for *Ephedra gerardiana* on the basis of RAPD analysis.

	MP1	TEG1	TEG2	TEG3	TEG4	TEG5	TEG6	TEG7	TEG8	TEG9
MP1	1									
TEG1	0.9	1								
TEG2	0.96	0.86	1							
TEG3	0.92	0.88	0.88	1						
TEG4	0.94	0.9	0.9	0.87	1					
TEG5	0.93	0.88	0.91	0.92	0.87	1				
TEG6	0.95	0.87	0.96	0.91	0.9	0.89	1			
TEG7	0.95	0.89	0.92	0.9	0.92	0.88	0.9	1		
TEG8	0.96	0.89	0.94	0.89	0.92	0.95	0.91	0.91	1	
TEG9	0.95	0.87	0.92	0.94	0.9	0.95	0.92	0.91	0.91	1

Table 4. List of primers exhibiting DNA amplification on the basis of SSR analysis of *Ephedra gerardiana*

S. No.	Marker	SSR Motif	Amplified band	Polymorphic band
1	F-AAGGTTAGCTTCAACATGGA R- AGACTCCATGAGTCCACAAC	(TC)11	1	-
2	F-AGCCCTAAGTAAGGCTCCTA R- TTCTCATCCTCTGTTTCCTC	(GCT)7	2	-
3	F- CTGCGTTTGAGAAATATCGT R- CCCTGAAGGTCAAGGAAAT	(GA)8	1	-
4	F- GGCTGAGAAGAGATACGAGA R- GCCTTAGGAAGTTCAGCTTT	(GTG)6	1	-
5	F- TTACAGATGGTACAGCCACA R- TGAAGATTTGCCACTCTCTT	(GA)7	1	-
6	F- CCAACATGTTCAACAATCTC R- TATCAAGCTTCCTTGTCCAT	(AG)6	3	1
7	F- CGTATTCTCCAAATTGCATC R- CAGATCAAGAAAGCGAAATC	(CGC)6	3	-
8	F- TTACAGATGGTACAGCCACA R- TGAAGATTTGCCACTCTCTT	(GA)22	2	-
9	F- CTCCAATGATAGACCCAGAA R- AGGATAACCTGATTGCTGTG	(GAT)6	1	-
10	F- GCTTGAGAAGAATCCAGATG R- AGCATGAAGTCTCTTCGTTT	(AGC)6	2	-
11	F- GGAGAACTTTCTGTCTCAAA R- AGTGTTGCTCAAAGAGCCTA	(AG)8	2	1
12	F- TTGGAATGAATCAACCTGAC R- GGGACAGTGTATCTCCTTGA	(TAG)6	1	-
13	F- GTATTCTCCAAATTGCATCC R- CAGATCAAGAAAGCGAAATC	(CG)6	1	-
14	F- TCATCAGCAACAGCAATATG R- CAATAAAGCAGATTCGTCGT	(CA)6	2	-
15	F- GCAGCATCATACTCAAATCC R- CCGGGTTTGTATCGTATATC	(CCT)6	2	-
16	F- TTAGCATCTGGTGATCCTGT R- AGATCCACTCCCTACGAAAT	(AGC)6	2	-
17	F- TCTTGAAACCCTAGAAACCA R- CTCCAACCTGAAGAAGAAGA	(GAG)6	3	1
18	F- CGGTGAATTGAGAGTGAAGA R- CGAAATCTAGACCTTTGTGG	(CAG)6	3	-
Total Number of Band			**33**	**03**

Table 5. Data of SSR analysis

S. No.	Parameters	Readings
1	Number of primers assay	18
2	Number of amplicon	33
3	Average Number of amplicon	1.83
4	Average number of polymorphic amplicon	0.09
5	Percentage polymorphism	9.09

Table 6. Nei's genetic similarity Jacard coefficient for *Ephedra gerardiana* on the basis of SSR primers

	MP1	TEG1	TEG2	TEG3	TEG4	TEG5	TEG6	TEG7	TEG8	TEG9
MP1	1									
TEG1	1	1								
TEG2	0.76	0.76	1							
TEG3	1	1	0.76	1						
TEG4	1	1	0.76	1	1					
TEG5	0.76	0.76	1	0.76	0.76	1				
TEG6	1	1	0.76	1	1	0.76	1			
TEG7	0.96	0.96	0.80	0.96	0.96	0.80	0.96	1		
TEG8	1	1	0.76	1	1	0.76	1	0.96	1	
TEG9	0.96	1	1	1	1	0.76	1	0.96	1	1

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RESEARCH ARTICLE

SCREENING OF NATIVE BACILLUS THURINGIENSIS (BT) ISOLATES FOR THE PRESENCE OF CRY 1 AB & *VIP 3A*

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Abstract: Insecticidal *cry* and *vip* genes from *Bacillus thuringiensis* (Bt) have been used for control of lepidopteran insects in transgenic crops. However, novel genes are required for gene pyramiding to delay evolution of resistance to the currently deployed genes.PCR-based techniques were employed for screening of *cry1Ab* type genes in 96 Bt isolates from diverse habitats in India and 8 known Bt strains. 96 native Bt isolates, recovered from different locations in India and 8 known Bt strains were screened for the presence of cry 1 Ab, cry 1Ac, Cry 3A & vip 3A for Isolation of plasmid DNA from native Bt isolates of Bacillus thuringiensis, Screening for the presence of cry 1 Ab, cry 1Ac, cry 3A & vip 3A gene using PCR amplification and Cloning of partial cry 1 Ab & vip 3A gene using different sets of primers. *Cry1Ab* type genes were more prevalent than *cry1Aa*- and *cry1Ac* type genes. Correlation between source of isolates and abundance of *cry1*-type genes was not observed..

Keywords: *Bacillus thuringiensis*, *Cry1Ab* genes, *Cry1Ac*, *Cry3A*, Vip*3A*, *Helicoverpa armigera*, Insecticidal genes

INTRODUCTION

Bacillus thuringiensis (Bt) is an aerobic, Gram-positive, spore-forming, facultative bacterial pathogen that produces parasporal crystals containing 1 or more insecticidal crystal proteins (Cry), which are selectively toxic to insects (Schnepf et al. 1998; van Frankenhuyzen 2013; Adang et al. 2014). Bt has been used as a biopesticide in agriculture, forestry, and mosquito control (Kaur 2000). Synthetic *cry* genes, modified for plant-preferred codon usage, have been transferred into crop plants for insect resistance (Koziel et al. 1993). Bt transgenic corn, cotton, tomato, potato, and soybean have been developed (James 2013). The search for novel cry and *vip* (vegetative insec- ticidal proteins) genes from native Bt isolates is an on- going effortworldwide (Kaur 2006). *Helicoverpa armigera* is a polyphagous pest of several crops, such as cotton, pigeon pea, chickpea, sorghum and tomato worldwide, which has developed resistance to chemical pesticides (Subramanian and Mohankumar 2006). Of the different types of *cry* genes, *cry1Ac* has been found to be most toxic towards *H. armigera* (Liao et al.2002). However, development of resistance in *H. armigera*, exposed to cry1Ac gene upon large-scale cultivation of first-generation transgenic crops, is a disconcerting possibility (Kaur 2012; Pardo López et al. 2013; Fabrick *et al.*2014). Gene pyramiding, deploying more than 1 type of insecticidal genes, having different receptors in the in- sect midgut, is the best strategy to delay the onset of resistance (Tabashnik et al. 2013; Carriere *et al.* 2015). *cry2*-type genes are promising candidates for gene pyra- miding owing to a difference in structure and mode of action In heliothine-type insects such as *H. armigera*, Cry2-type proteins have been shown to have binding sites different from those of other Cry proteins deployed in transgenic crops such as Cry1Ac, Cry1F, and Vip3A proteins (Gouffon et al. 2011). *cry1Ac*-resistant *H. armigera* did not show cross resistance to *cry2Aa* (Akhurst et al. 2003). Furthermore, a synergistic effect from the combination of Cry1Ac and Cry2Ab proteins has been observed towards *H. armigera* (Ibargutxi et al. 2008).

Novel *cry2*-type genes have been identified by PCR and cloned in many laboratories in India and elsewhere (Misra et al. 2002; Sauka et al. 2005; Beard et al. 2008; Lin et al. 2008; Shu et al 2013; Somwatcharajit et al. 2014). PCR- based identification of *cry* genes was first developed by Carozzi et al. (1991) and has remained the method of choice because of its sensitivity, rapidity, and ease of performance (Porcar and Juarez-Perez 2003). In this study, 96 native Bt isolates, recovered from different locations in India (Table 1), and 8 known Bt strains (Table 2) were screened for the presence of cry 1 Ab, cry 1Ac, Cry 3A & vip 3A with the objectives of .Isolation of plasmid DNA from native Bt isolates of Bacillus thuringiensis, Screening for the presence of cry 1 Ab, cry 1Ac, cry 3A & vip 3A gene using PCR amplification and Cloning of partial cry 1 Ab & vip 3A gene using different sets of primers.

*Corresponding Author

MATERIAL AND METHOD

Bacterial strain: 98 Native Bt isolates recovered from diverse agricultural & Non agricultural location of India. These strain were provided by Dr. Sarvjeet Kaur Laboratory NRCPB. IARI New Delhi

The objective of the study was screening of native Bt isolates for the presence of cry 1Ab & vip 3A gene. This could be used as a promising tool for the effective control and resistant management of agricultural important species of insect pests. The methodology includes:

- Overnight grown Culture of *Bacillus thuringiensis* was prepared using LB containing appropriate amount of penicillin at 28^0c- 30^0c.
- The bacterial plasmid DNA from overnight grown culture of bacillus thuringiensis was isolated by alkali lyses method.
- The plasmid was observed as a discrete band of size ~23kb on agarose gel electrophoresis.
- Since cry gene is plasmid encoded, therefore the isolated plasmids were further screened for the presence of cry gene.
- The most common method utilized for screening was polymerase chain reaction (PCR). Set of gene specific primers were used to detect and amplify the cry 1Ab & *vip* 3A gene.
- The PCR product was observed on 0.8% Agarose gel electrophoresis as discrete band.
- The band with desired gene size was finally cut under UV transilluminator and kept at-200C.
- Competent cells of Ecoli DH5a were prepared.
- The band was further eluted out and ligated in a pDrive vector.
- Transformation was carried out by incubating competent cells with ligation mixture.
- Transformed cells were then plated onto LA+ ampicillin + X-gal- IPTG plates.
- Plates were screened for white colonies.
- Plasmid was isolated from these transformed colonies and screened for the presence of cry gene.

RESULT AND DISCUSSION

Molecular characterization: plasmid DNA was isolated from 88 native Bt isolates (Table 1) & 8 Bt reference strain (Table 2). The extracted DNA was analyzed by the PCR with specifically designed primer.

Isolation of Plasmid DNA: plasmid DNA of native strain showed a band of ~23kb in size.

PCR screening of natives Bt isolates for presence of full length cry 1Ab gene.

PCR amplification was carried out with primer 3 & 4 corresponding to full length cry 1Ab gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	3 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	4 min.	
$MgCl_2$	-	2.5ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	1ul				
D/H_2o	-	9.5ul				

The Full length of cry 1 Ab gene was not observed in any isolates, however, shorter PCR products of different size were observed in some isolates. (Table 3).

PCR screening of natives Bt isolates for presence of full length cry 1Ab gene.

PCR amplification was carried out with primer 110&111 corresponding to full length vip 3A gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	3 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	4 min.	
$MgCl_2$	-	3.0ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	0.5ul				
D/H_2o	-	9.5ul				

The Full length of vip 3A gene was not observed in any isolates, however, shorter PCR products of different size were observed in some isolates.(Table 4)

PCR screening of natives Bt isolates for presence of full length cry 3A gene.

PCR amplification was carried out with primer 63&64 corresponding to full length cry 3A gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	3 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	4 min.	
$MgCl_2$	-	3.0ul	72^0C	-	10 min	

PCR buffer	-	2.5ul
Taq. Polymerase	-	0.5ul
D/H_2o	-	9.5ul

The Full length of cry 3A gene was not observed in any isolates, however, shorter PCR products of different size were observed in some isolates.(Table 5)

PCR screening of natives Bt isolates for presence of full length cry 3A gene.

PCR amplification was carried out with primer 114&115 corresponding to full length cry 3A gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	2 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	2 min.	
$MgCl_2$	-	3.0ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	0.5ul				
D/H_2o	-	9.5ul				

The Full length of cry 3A gene was not observed in any isolates, however, shorter PCR products of different size were observed in some isolates. (Table 6)

PCR screening of natives Bt isolates for presence of full length cry 1 Ac gene.

PCR amplification was carried out with primer 112&113 corresponding to full length cry 1 Ac gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	3 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	4 min.	
$MgCl_2$	-	2.5ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	1.0ul				
D/H_2o	-	9.5ul				

The Full length of cry 1 Ac gene was not observed in any isolates, however, shorter PCR products of different size were observed in some isolates (Table 7).

PCR screening of natives Bt isolates for presence of full length vip 3A gene.

PCR amplification was carried out with primer 1&2 corresponding to full length vip 3A gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	2 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	2 min.	
$MgCl_2$	-	3.0ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	0.5ul				
D/H_2o	-	9.5ul				

The Full length of vip 3A gene was observed in 4N1, T-55, 986, 4J2 isolates. (Table 8)

PCR screening of natives Bt isolates for presence of full length vip 3A gene.

PCR amplification was carried out with primer 1&3 corresponding to full length vip 3A gene.

Reaction mixture			**PCR condition**			
DNA	-	5ul	94^0C	-	2 min.	
Primer (f)	-	1ul	94^0C	-	1 min.	30 cycle
Primer (r)	-	1ul	40^0C	-	1 min.	
dNTP	-	2.5ul	72^0C	-	2 min.	
$MgCl_2$	-	3.0ul	72^0C	-	10 min	
PCR buffer	-	2.5ul				
Taq. Polymerase	-	0.5ul				
D/H_2o	-	9.5ul				

The expected size of 2.37 kb was observed in 4A6 reference of native Bt isolates. (Table 9)

Table 1. List of native Bt strain isolates from different location of India.

S.No.	Strain no	Plasmid DNA		S. No.	Strain No.	Plasmid DNA		S. No.	Strain No.	Plasmid DNA		S.No.	Strain No.	Plasmid DNA	
		23kb	48kb			23kb	48kb			23kb	48kb			23kb	48kb
1	1	+	-	23	220	+	-	45	784	+	-	67	922	+	-
2	3	+	-	24	222	+	-	46	791	+	-	68	935	+	-

3	4	+	-	25	223	+	-	47	792	+	-	69	980	+	-
4	5	+	-	26	229	+	-	48	700	+	-	70	942	+	-
5	9	+	-	27	232	+	-	49	701	+	-	71	945	+	-
6	13	+	-	28	301	+	-	50	707	+	-	72	948	+	-
7	20	+	-	29	302	+	-	51	721	+	-	73	949	+	-
8	28	+	-	30	303	+	-	52	722	+	-	74	950	+	-
9	48	+	-	31	304	+	-	53	727	+	-	75	951	+	-
10	51	+	-	32	305	+	-	54	729	+	-	76	952	+	-
11	63	+	-	33	307	+	-	55	739	+	-	77	954	+	-
12	82	+	-	34	611	+	-	56	741	+	-	78	955	+	-
13	84	+	-	35	608	+	-	57	758	+	-	79	956	+	-
14	88	+	-	36	629	+	-	58	754	+	-	80	957	+	-
15	94	+	-	37	668	+	-	59	753	+	-	81	958	+	-
16	110	+	-	38	669	+	-	60	783	+	-	82	959	+	-
17	113	+	-	39	678	+	-	61	793	+	-	83	960	+	-
18	208	+	-	40	680	+	-	62	794	+	-	84	961	+	-
19	213	+	-	41	751	+	-	63	807	+	-	85	977	+	-
20	214	+	-	42	763	+	-	64	911	+	-	86	980	+	-
21	217	+	-	43	767	+	-	65	918	+	-	87	986	+	-
22	219	+	-	44	772	+	-	66	918	+	-	88	995	+	-

Table 2. List of Reference Strain Bt strain isolates from different location of India.

S.No.	Strain no. (88 Samples)	Plasmid DNA	
		23 kb	48 kb
1	4A6	+	-
2	4M2	+	-
3	4K1	+	-
4	4F1	+	-
5	4J2	+	-
6	4K1	+	-
7	4R1	+	-
8	4S2	+	-
Total 8			

Table 3. List of PCR amplification for full length cry 1 Ab gene with primer 3&4

S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	-		23	302	-		45	772	-		67	957	-	
2	3	-		24	303	-		46	783	-		68	958	-	
3	4	-		25	304	-		47	671	-		69	959	-	
4	9	-		26	305	-		48	792	-		70	960	-	
5	13	-		27	307	-		49	793	-		71	961	-	
6	20	-		28	611	-		50	807	-		72	977	-	
7	28	-		29	618	-		51	851	-		73	986	-	
8	32	-		30	629	-		52	911	-		74	995	-	
9	51	-		31	668	-		53	916	-		75	996	-	
10	63	-		32	678	-		54	918	+(2kb)		76	4M2	-	
11	82	-		33	680	-		55	922	-		77	4A6	-	
12	84	-		34	700	+ (1kb)		56	935	-		78	4W1	+(500bp)	
13	88	-		35	701	-		57	980	-		79	4M2	+(>1kb)	
14	94	-		36	721	-		58	944	+(2kb)		80	4R1	-	
15	110	-		37	792	-		59	945	-		81	4K1	+(2.0kb)	
16	113	-		38	729	+(1.5 kb)		60	948	-		82	4F1	-	
17	217	-		39	739	-		61	949	+(250bp)		83	4S2	-	
18	219	-		40	750	-		62	950	+(2kb)		84	T-55	-	
19	220	-		41	751	-		63	951	-		85	4C3	-	
20	222	-		42	753	-		64	952	-		86	4L3	-	
21	223	-		43	754	-		65	954	+(2kb)		87	T-94	+(250)bp	
22	301	-		44	758	-		66	955	-					

Table 4. List of PCR amplification for full length vip 3A gene with primer 110 & 111

S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	-		23	302	-		45	758	-		67	957	-	
2	3	-		24	303	-		46	772	-		68	958	-	
3	4	-		25	304	-		47	783	-		69	959	-	
4	9	-		26	305	-		48	671	-		70	960	-	
5	13	-		27	306	-		49	792	-		71	961	-	
6	20	-		28	307	-		50	793	-		72	977	-	
7	28	-		29	611	-		51	807	-		73	986	-	
8	32	-		30	618	-		52	911	-		74	995	-	
9	51	-		31	629	-		53	916	-		75	996	-	
10	63	-		32	668	-		54	918	-		76	4M2	+(1.2kb)	
11	82	-		33	678	-		55	922	-		77	4A6	-	
12	84	-		34	680	-		56	935	-		78	4W1	-	

13	88	-		35	700	-		57	980	-		79	4K1	-	
14	94	-		36	701	-		58	944	-		80	4C3	-	
15	110	-		37	721	-		59	945	-		81	4L3	-	
16	113	-		38	792	-		60	948	-		82	4F1	-	
17	217	-		39	729	-		61	949	-		83	4R1	-	
18	219	-		40	739	-		62	950	-		84	T-55	-	
19	220	-		41	750	-		63	951	-		85	T-94	-	
20	222	-		42	751	-		64	952	-		86	T-15	-	
21	223	-		43	753	-		65	954	-					
22	301	-		44	754	-		66	955	-					

Table 5. List of PCR amplification for full length cry 3A gene with primer 63& 64

S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	1	-	25	304	-		49	918	-		73	995	-	
2	2	3	-	26	305	-		50	922	-		74	996	-	
3	3	4	-	27	307	-		51	935	-		75	4M2	-	
4	4	9	-	28	611	-		52	955	-		76	4A6	+(1.2kb)	
5	5	13	-	29	618	-		53	936	-		77	4W1	-	
6	6	20	-	30	629	-		54	951	-		78	4R1	-	
7	7	28	-	31	668	-		55	950	-		79	4K1	-	
8	8	32	-	32	678	-		56	957	-		80	4F1	-	
9	9	51	-	33	680	-		57	960	-		81	4S2	-	
10	10	63	-	34	700	-		58	700	-		82	4J2	-	
11	11	82	-	35	701	-		59	749	-		83	4N1	-	
12	12	84	-	36	721	-		60	T-94	-		84	T-55	-	
13	13	88	-	37	722	-		61	4M2	-		85	4C3	-	
14	14	94	-	38	792	-		62	942	-		86	4L3	-	
15	15	110	-	39	729	-		63	952	-		87	T-94	-	
16	16	113	-	40	739	-		64	954	-		88	T-27	-	
17	17	217	-	41	750	-		65	955	-		89	942	-	
18	18	219	-	42	751	-		66	956	-		90	4K1	-	
19	19	220	-	43	753	-		67	977	-		91	4L3	-	
20	20	222	-	44	783	-		68	958	-		92	956	-	
21	21	223	-	45	792	-		69	959	-		93	4J2	-	
22	22	301	-	46	772	-		70	960	-		94	4K1	-	
23	23	302	-	47	793	-		71	977	-		95	4S2	-	
24	24	303	-	48	851	-		72	986	-		96	4L3	-	

Table 6. List of PCR amplification for full length cry 3A gene with primer 114& 115

S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	-		26	305	-		51	851	-		76	948	-	
2	3	-		27	307	-		52	911	-		77	4M2	-	
3	4	-		28	611	-		53	916	-		78	4A6	-	
4	9	-		29	618	-		54	918	-		79	4W1	-	
5	13	-		30	629	-		55	922	+(750bp)		80	4W1	-	
6	20	-		31	668	-		56	935	+(750bp)		81	4R1	-	
7	28	-		32	678	-		57	980	+(300bp)		82	4M2	-	
8	32	-		33	680	-		58	944	+(250bp)		83	4W1	-	
9	51	-		34	700	-		59	945	-		84	942	-	
10	63	-		35	701	-		60	948	-		85	4K1	-	
11	82	-		36	721	-		61	949	-		86	4F1	-	
12	84	-		37	792	-		62	950	-		87	4S2	-	
13	88	-		38	729	+(1.2kb)		63	951	-		88	4J2	-	
14	94	-		39	739	-		64	952	-		89	4N1	-	
15	110	-		40	750	-		65	954	-		90	T-55	-	
16	113	-		41	751	-		66	955	-		91	4C3	-	
17	217	-		42	753	-		67	957	-		92	4L3	-	
18	219	-		43	754	-		68	958	-		93	T-94	-	
19	220	-		44	758	-		69	959	-		94	4S2	-	
20	222	-		45	772	-		70	960	-		95	4K1	-	
21	223	-		46	793	-		71	961	-		96	4C3	-	
22	301	-		47	671	-		72	977	-		97	4L3	-	
23	302	-		48	792	-		73	977	-		98	T-94	-	
24	303	-		49	793	-		74	986	-		99	4J2	-	
25	304	-		50	807	-		75	995	-		100	4K1	-	

Table 7. List of PCR amplification for full length cry 1 Ac gene with primer 112&113

S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products		S.No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	-		25	304	-		49	807	-		73	4J3	-	
2	3	-		26	305	-		50	669	-		74	986	+(1.7kb)	
3	4	-		27	405	-		51	911	-		75	995	+(1.1kb)	
4	9	-		28	611	-		52	916	-		76	948	-	
5	680	-		29	618	-		53	918	-		77	4M2	-	

6	20	-		30	729	-		54	922	-		78	4A6	-	
7	20	-		31	668	-		55	935	-		79	4M2	-	
8	918	-		32	669	-		56	980	-		80	T-27	-	
9	51	-		33	678	-		57	942	+(500bp)		81	4K1	-	
10	63	-		34	680	-		58	945	+(250bp)		82	4M2	-	
11	82	-		35	700	-		59	948	-		83	4W1	-	
12	84	-		36	701	-		60	949	+(500bp)		84	942	-	
13	88	-		37	721	-		61	950	-		85	4K1	-	
14	94	-		38	792	+(1.2kb)		62	951	+(1kb)		86	4F1	-	
15	110	-		39	729	-		63	952	-		87	952	-	
16	113	-		40	739	-		64	954	-		88	4J2	-	
17	217	-		41	750	-		65	955	+(1kb)		89	4N1	-	
18	219	-		42	751	-		66	956	-		90	T-55	-	
19	T-94	-		43	753	-		67	957	+(1.7,1.1,.8kb)		91	4L3	-	
20	222	-		44	754	-		68	958	+(1.7,1.1,.8kb)		92	4R1	-	
21	223	-		45	758	-		69	959	-		93	4J2	-	
22	301	-		46	671	-		70	960	-		94	4K1	-	
23	302	-		47	792	-		71	961	-		95	4L3	-	
24	303	-		48	793	-		72	977	-		96	4C3	-	

Table 8. List of PCR amplification for full length vip 3A gene with primer 1&2

S. No.	Bt Isolates	PCR Products		S. No.	Bt Isolates	PCR Products		S. No.	Bt Isolates	PCR Products		S. No.	Bt Isolates	PCR Products	
		(+)	(-)			(+)	(-)			(+)	(-)			(+)	(-)
1	1	-		26	305	-		51	1			76	986	+(400bp)	
2	3	-		27	405	-		52	669			77	995		
3	4	-		28	954	-		53	911			78	110		
4	9	-		29	618			54	916			79	4M2		
5	680	-		30	792			55	918			80	4A6	+(400bp)	
6	20	-		31	668			56	918			81	729		
7	20	-		32	669			57	303			82	T-27		
8	110	-		33	678			58	935			83	110		
9	51	-		34	680			59	980			84	950	+(400bp)	
10	63	-		35	700			60	942			85	4W1		
11	82	-		36	701			61	959			86	739	+(400bp)	
12	84	-		37	721			62	948			87	4K1		
13	88	-		38	792			63	949			88	4F1		
14	94	-		39	729			64	950			89	952	+(500bp)	
15	110	-		40	739			65	950			90	492	+(400bp)	
16	113	-		41	750			66	952			91	4J2	+(400bp)	
17	217	-		42	751			67	954			92	4M1	+410bp	
18	219	-		43	753			68	955			93	T-55	+410bp	
19	T-94	-		44	754			69	962			94	4L3		
20	680	-		45	758			70	945			95	4R1		
21	223	-		46	671			71	958			96	4A6		
22	301	-		47	783			72	977			97	4J2		
23	302	-		48	671			73	960			98	4K1		
24	303	-		49	792			74	977						
25	304	-		50	793			75	452	+(400bp)					

Table 9. List of PCR amplification for full length vip 3A gene with primer 1&3

S.No.	Bt Isolates	PCR Products	
		(+)	(-)
1	4A6	+(2.37kb)	
1	4A6	+(2.37kb)	
2	4J2	-	
3	4J2	-	
4	986	-	
5	995	-	
6	4M2	-	
7	4A6	+(2.37kb)	
8	950	-	
9	739	-	
10	952	-	
11	4N1	-	
12	J-55	-	
13	4L3	-	
14	20	-	
15	20	-	
16	110	-	
17	63	-	
18	T-94	-	
19	301	-	
20	680	-	

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RESEARCH ARTICLE

RESPONSE OF SORGHUM [*SORGHUM BICOLOR* (L.) MEONCH] GENOTYPES TO DIFFERENT FERTILITY LEVELS ON NUTRIENT UPTAKE, AVAILABLE SOIL NUTRIENTS AFTER HARVEST AND YIELDS

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Abstract: An experiment was conducted at the Instructional Farm, Rajasthan College of Agriculture, during *kharif* 2012. To study the effect of different fertility levels on nutrient uptake and nutrient status in soil nutrients after the harvest and yields of crop. Four fertility levels *i.e*. control, 50, 75 and 100% RDF (recommended dose of fertilizers; 80 kg N+40kg P_2O_5 +40kg K_2O ha^{-1}) and 6 elite sorghum genotypes (SPH 1674, SPH 1680, SPV 2083, CSH 16, CSH 25 and CSV 23) were compared in a factorial randomized block design. Maximum nitrogen uptake by grain, maximum protein uptake by grain, as well as fodder, with genotype SPH 1674. CSV 23 recorded maximum phosphorus uptake (22.66 ha^{-1}) by fodder. Results showed that application of 100 % RDF gave significantly higher grain, fodder and biological yields over 50 % and control.Significantly increased available N, P & K contents in soil after harvest the sorghum crop over control.CSV 23 and SPV 2083 recorded significantly maximum available N, P and K in soil after harvest over rest of the genotypes.SPH 1674 recorded significantly higher grain yield (61.94 q ha^{-1}) and harvest index (34.48 %) than other genotypes.

Keywords: Fertility levels, Genotypes, Nutrient uptake, Available soil after harvest, Yield

INTRODUCTION

Sorghum [*Sorghum bicolor* (L.) Munch] is the king of millets and third important crop in the country after rice and wheat. Sorghum is not only staple food but it is also required to fulfill fodder requirement in order to make animal husbandry sector more viable. There is a great need to maintain regular well balance supply of more nutritious feed and fodder in the state. The quick spreading of high yielding genotypes changed the scenario of sorghum production in India. Now at present India produces that much amount of sorghum grain that we produced just double acreage during 1950,s. These are not a dream but it was achieved only by using high yielding and fertilizer responsive cultivars of sorghum. While, the need for adequate fertilizer requirement is desirable, where as identification of suitable genotype with genetic potential is equally important. Sorghum is a highly nutrient exhaustive crop and the importance of nitrogen and phosphorus in its nutrition is well documented. Thus, suitable cultivars and proper nutrition are very important to achieve higher yield. Hence, the present study was undertaken to find out the response of different elite sorghum genotypes to fertility levels.

MATERIAL AND METHOD

A field investigation was carried out during the *kharif* 2012 at the Instructional Farm, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture and Technology, Udaipur which is situated at 23°34' N latitude, 73°42' E longitude and at an altitude of 582.17 meters above the mean sea level, to study the effect of various fertility levels on growth and yield of elite sorghum cultivars. The maximum and minimum temperature during sorghum crop growth period ranged between 28.1 ºC to 36.3 ºC and 15.4 ºC to 26.6 ºC, respectively. The soil of experimental site was clay loam in texture having slightly alkaline pH (8.0) in reaction, organic carbon (0.77 %), medium with respect to available nitrogen (292.50 kg ha^{-1}), available phosphorus (20.25 kg ha^{-1}) and high in available potassium (198.65 kg ha^{-1}). The experiment consisting of 24 treatment combinations comprising four fertility levels [control, 50, 75 and 100 % RDF (recommended dose of fertilizer; 80 kg N+40kg P_2O_5 +40kg K_2O ha^{-1}) and six elite sorghum genotypes (SPH 1674, SPH 1680, SPV 2083, CSH 16, CSH 25 and CSV 23) were compared in a factorial randomized block design having three replications. Sorghum genotypes were sown on 4 July, 2012 at 45x 12-15 cm row to row and plant to plant spacing with a seed rate of about 10 kg ha^{-1}.Half dose of nitrogen of each level and full doses of P_2O_5 and K_2O will be applied as basal dose at sowing and remaining half dose of nitrogen will be applied crop at 30 DAS by broadcasting through urea in standing crop. Total rainfall received during crop growing season was 642.40 mm. Crop was harvested on 15 October, 2012. For grain yield, earheads from each net plot were picked up and kept in gunny bags. After through sun drying these were threshed, winnowed and cleaned. After sun drying 2-3 days the grain weight of individual plot was recorded and final yield was expressed in kg ha^{-1}, while after detaching the earheads the fodder was left in the field for sun drying for few days. After drying, the bundles

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of each plot were weight and noted dry fodder yield per unit area. The harvest index calculated by dividing the economic yield (grain yield) by biological yield and expressed in percentage (Donald and Hamblin, 1976).

RESULT AND DISCUSSION

A perusal of data presented in Table 1 reveals that application of fertility levels significantly enhanced N uptake by grain, fodder and total uptake by crop over control. Among the fertility levels ever increase in fertility levels from 0 to 100 % RDF significantly increased N uptake by all parts i.e. grain as well as fodder and total uptake by crop except in grain where significant response was noted only up to 75 % RDF. The increased with the 100 % RDF were by 50.67, 20.42 & 5.18 per cent in grain 52.51, 19.77 & 10.80 per cent in fodder and 51.43, 20.15 and 7.46 per cent in total N uptake by crop over control, 50 and 75 % RDF, respectively.

The data presented in table 1 reveals that application of fertility levelssignificantly improved P uptake by grain, fodder and total uptake by crop over control and maximum was noted in 100 % RDF in all the parameters i.e. grain, fodder and total uptake by crop over both the lower doses i.e. 50 and 75 % RDF except P uptake by grain whereas significant response was noted only up to 75 % RDF over 50 % RDF. The increases with the 100 % RDF were to the tune of 52.51, 23.25 & 5.10 per cent in grain, 41.37, 13.11 & 8.72 per cent in fodder and 45.66, 17.08 & 7.13 per cent in total P uptake by crop over control, 50 and 75 % RDF, respectively.

A perusal of data presented in Table 1 reveals that application of fertility levelssignificantly improved K uptake by grain, fodder and total uptake by crop over control. Among the levels of fertility significantly maximum was noted with the application of 100 % RDF in all the parameters i.e. grain, fodder and total uptake by crop over both the lower doses i.e. 50 and 75 % RDF except K uptake by grain whereas significant response was noted only up to 75 % RDF over 50 % RDF. The increases with the 100 % RDF were to the tune of 57.34, 25.90 & 6.03 per cent in grain, 45.84, 18.70 & 12.03 per cent in fodder and 47.10, 19.51 & 11.29 per cent in total K uptake by crop over control, 50 and 75 % RDF, respectively.

The total uptake ofN,P and K by sorghum was increased significantly due to fertilizer application. The maximum uptake ofN (167.27 kg ha^{-1}), P (40.72 kg ha^{-1}) and K (259.69 kg ha^{-1}) at harvest recorded with application of 100% RDF. It is an established fact that accumulation of nutrients is dependent on their concentration at cellular level and dry matter production. Thus, impact of N,P and K fertilizer on their ultimately led to higher accumulation of nutrients by plant parts along with total uptake by the crop. Application of fertility levelssignificantly enhanced proteinuptake by grain since the protein content is computed from nitrogen content of grain which has enhanced under the above said treatments because of increased availability of soil nitrogen. Result with the findings of Das *et al*. (2000), Kaushik (2000), Jat*et al*. (2003), Sumeriya *et al*. (2005), Dixit *et al*. (2005), Dhaker (2010) and Sumeriya (2010).

A perusal of data presented in Table 1 reveals that application of fertility levelssignificantly enhanced proteinuptake by grain, fodder and total uptake by crop over control. Among the fertility levels protein uptake by grain was significantly responded up to 75 % RDF over 50 % RDF but further, increase in fertility from 75 % to 100 % RDF failed to increase protein uptake by grain. Whereas in case of protein uptake in fodder and total uptake by the crop, ever increase in fertility from 0 to 100 % RDF obtained significantly higher protein uptake. The increases with the 100 % RDF were to the tune of 52.51, 19.78 and 10.81 protein in fodder, 51.37, 20.15 and 7.46 per cent in total uptake over control, 50 and 75 % RDF, respectively.

Application of fertility levelssignificantly enhanced proteinuptake by grain since the protein content is computed from nitrogen content of grain which has enhanced under the above said treatments because of increased availability of soil nitrogen. An increase in protein content as a consequence of nitrogen, phosphorous and potassium fertilization is in agreement with the findings of Das *et al*. (2000), Kaushik (2000), Jat*et al*. (2003), Sumeriya *et al*. (2005), Dixit *et al*. (2005), Dhaker (2010) and Sumeriya (2010).

A perusal of data presented in Table 2 reveals that application of fertility levels significantly increased available N, P & K contents in soil after harvest the sorghum crop over control. While, among the levels, 100 % RDF provided significantly maximum N, P &K content over 50 % RDF but 75 % RDF was at par with higher as well as lower i.e. 100 & 50 % RDF in all nutrients content in soil.

The status of availability of N in the soil after harvesting of sorghum was lower than the initial status of the soil under all the treatments due to the fact that sorghum crop is a heavy feeder ofN. At higher doses of nitrogen, phosphorus and potassium the crop growth was also better than their lower doses. So naturally more uptake of nitrogen took place under these treatments. However, the available status of phosphorus and potassium in soil at harvest was observed with increasing fertility levels might be due to build up of nutrients in soil as a result of addition ofN,Pand K. Besides, on addition of fertilizer P to the soils, there might be some sort of triggering action on native soil P resulting in increased availability. Vaidya and Gabhane (1998) and Patil and Varade (1998) also reported an increase in nutrient availability after harvest of sorghum with increasing rate of fertilizer application.

It can be inferred from data presented in Table 3 that application of fertility levels significantly increased

the grain, dry fodder and biological yields over no fertility (control). Among the levels of fertility significant response was noted this application of 75 % RDF in grain production over 50 % RDF while, further increased in fertility from 75 to 100 % RDF was failed to show any significant response in grain yield. The increases with 75 % RDF were to the tune of 38.62 and 14.55 per cent over control and 50 % RDF.

But in case of dry fodder and biological yield application of 100 % RDF provided significantly maximum yields over both the lower doses. While, application of 75 % RDF also proved significantly superior over 50 % RDF in both dry fodder and biological yields. The increases with the 100 % RDF were by 34.80, 12.14 & 8.27 per cent in dry fodder and 37.55, 14.21 & 6.98 per cent in biological yield over control, 50 & 75 % RDF, respectively.

The significant increases in fodder yield with increasing fertility levels could be attributed to conducive effect on root and shoot growth of the plant which in turn has accrued from increased plant height and dry matter accumulation. The correlation studies also substantiate significant positive relationship between fodder yields. The profound influence of fertilizer on biological yield seems to be an account of its influence on vegetative and reproductive aspects of crop growth. The results of present investigation are in close agreement with the findings of Kushwaha and Thakur (2006), Singh and Sumeriya (2006) Dhaker (2010), Panwar*et al.* (2014) and Panwar*et al.* (2015).

A perusal of data presented in Table 3 reveals that application of fertility levels significantly improved the harvest index. While, all the levels of fertility did not prove their superiority with one another in this respect.

A perusal of data presented in Table 3 indicates that application of 100 % RDF provided significantly higher gross, net returns and B/ C ratio over all the lower doses whereas ever increase in fertility 0 to 100 % RDF increased the gross and monetary returns. But in case of B/ C ratio response was obtained only upto 75 % RDF. The increases with 100 % RDF were to the tune of 41.59, 17.23 and 5.23 per cent in gross and 50.77, 20.26 and 5.53 per cent net returns over control, 50, and 75 % RDF, respectively.

The increasing levels of fertility increased the gross and net returns (Table 3) up to 75 % RDF however, variation between 75 % RDF was found at par with 100 % RDF. Similar observation was also made by Singh and Sumeriya (2006), Dhaker (2010) and Kumawat (2013) on maize crop.

Further, data presented in Table 1 indicated that SPH1674 recorded significantly maximumnitrogen uptake by grain and total uptake by the crop over rest of the genotypes. GenotypeSPV 2083 and CSV 23 were estimated significantly minimum in N uptake and remaining were at par in both these parameters. But in case of fodder, variety CSV 23 provided significantly maximum Nuptake over rest of the genotypes except SPV 2083 which was at par. Remainings were statistically at par in this parameter.

An examination of data presented in Table 1 reveals that SPH1674 recorded significantly maximumphosphorus uptake by grain over rest of the genotypes. Whereas, genotypesCSV 23 and SPV 2083 produced significantly minimum P uptake by grain, remains were at par with one another SPH 1674 provided 13.97 & 14.94 per cent higher P uptake over CSH 16 and CSH 25,respectively.Further, data reveals that CSV 23 recorded maximumphosphorus uptake (22.66 kg ha^{-1}) by fodderwhich was remained at par withSPV 2083 and significantly superior over rest of the genotypes. Remaining genotypes were also noted at par with one another.However, SPH1674 recorded maximum total potassium uptake by cropsignificantly superior over rest of the genotypes.While, both the varieties were statistically poorer in this respect. Genotype SPH1674 produced 6.75, 14.81, 7.62, 6.21 and 12.97per cent higher total potassium uptake by cropover SPH1674,SPV 2083, CSH 16,CSH25 and CSV 23**,** respectively.

An examination of data presented in Table 1 reveals that SPH1674 recorded significantly maximum potassiumuptake by grain over rest of the genotypes. GenotypeSPH 1674 produced 11.57, 63.76, 6.91, 8.56 and 63.67 per cent higherpotassium uptake by grainoverSPH 1680, SPV 2083,CSH 16, CSH25 and CSV 23,respectively. Whereas, both the varieties i.e. SPV 2083 and CSV 23 were noted significantly poorer over rest of the genotypes in this respect.However, CSV 23 provided maximum potassium uptake by fodder (209.68 kg ha^{-1}) which was remained at parwithSPV 2083 and both were statistically superior over rest of the genotypes.In case of total K uptake by crop all the genotypes were failed to show any significant response with one another.

Nutrients (N,P and K) uptake increased with genotypes. As the uptake is a product of the yield and nutrient content, considerable increase in either of components may increase the uptake. The uptake of nutrients is mainly governed by the variation in grain and stover yield and their nutrient concentrations. Genotypic variation in uptake by grain, stover and total uptake of nitrogen, phosphorusand potassium by crop was significantly affected due to genotypes. However, comparing different genotypes for N, P and K nutrients, genotype SPH 1674 recorded highest N and P uptake by grain and total uptake by crop. While, obtained highest N and P instover and total K uptake by the crop in CSV 23. The improvement in protein content of grain of variety SPH 1674 seems to be on account of increased N concentration in grain. It is well known fact that N is a constituent of protein, enzymes and chlorophyll

and participates in several biochemical processes for the metabolism of carbohydrate and protein system. Thus higher protein might be on account of higher N content compared to other varieties. Genetic variability in sorghum with respect to plant nutrients uptake ofN,P and K were also observed by Dixit *et al*. (2005), Sumeriya *et al*. (2005), Dhaker (2010), Sumeriya (2010) and Mawaliya (2012).

The analytical data in Table 1 indicated that SPH1674 recorded significantly maximumprotein uptake in grainover rest of the genotypes. GenotypeSPV 2083 and CSV 23 provided significantly minimum protein uptake and responses were at par with another in this contrast. Genotypes SPH 1674 provided 11.17 and 12.09 per cent high protein uptake by grain over CSH 16 and CSH 25. In case of dry fodder significantly maximum protein uptake was obtained by test variety CSV 23 except SPV 2083 which was at par. Remains were also at par with one another. Further, data showed that genotypes SPH 1674 estimated significantly maximum total uptake by the crop whereas both the varieties i.e. SPV 2083 and CSV 23 were significantly poorer in this respect. Remains were at par with one another. The increased with SPH 1674 were by 8.52 and 8.16 per cent proved over CSH 16 and CSH 25, respectively.

The improvement in protein content of grain of variety SPH 1674 seems to be on account of increased N concentration in grain. It is well known fact that N is a constituent of protein, enzymes and chlorophyll and participates in several biochemical processes for the metabolism of carbohydrate and protein system. Thus higher protein might be on account of higher N content compared to other varieties. Result with the findings of Dixit *et al*. (2005), Sumeriya *et al*. (2005), Dhaker (2010), Sumeriya (2010) and Mawaliya (2012).

An examination of data presented in Table 2 reveals that CSV 23 recorded significantly maximum available nitrogen in soil after harvest over rest of the genotypes. GenotypeCSV 23 produced9.79, 5.52, 3.17, 4.76 and 3.27per cent maximumavailable nitrogen in soil after harvest overSPH1674, SPH 1680, SPV 2083,CSH 16 andCSH25,respectively. This was followed by SPV 2083 and CSH 25 which were proved significantly superior over SPH 1674.Further, data showed that CSV 23 recorded maximumavailable phosphorus in soil after harvestwhich was remained at par withSPV 2083, CSH 16andCSH25 and all these genotypes brought about significant inference over rest of the genotypes.

Whereas, SPV 2083 obtainedmaximumavailable potassium in soil after harvestwhich was remained at par withCSV 23 and significantly superior over rest of the genotypes.While, genotype CSV 23 noted significantly superior over SPH 1674 and at par with rest of the genotypes tested.

The status of available nitrogen, phosphorus and potassium in soil after harvesting of sorghum was lower than the initial status of soil under all the treatments due to the fact that sorghum crop is a heavy feeder of nitrogen, phosphorus and potassium. Sorghum genotypes CSV 23 left maximum amount of nitrogen, phosphorous and potassium (269.27, 21.36 and 357.97 kg $ha.^{-1}$) and SPH 1674 left minimum amount of nitrogen, phosphorous and potassium (245.24, 19.02 and 347.65 kg $ha.^{-1}$) to be soil after harvest the sorghum crop.

A critical examination of data in Table 3 reveals that SPH 1674 recorded significantly maximum grain yield (61.94 q ha^{-1})over rest of the genotypes. Genotype SPH 1674 produced 8.90, 9.74 and 55.71 per cent higher grain yield over CSH 16, CSH 25 and CSV 23,respectively. Further, SPH 1680 proved statistically at par with test hybrid CSH 16 and CSH 25. All these were brought about significant response over genotype SPV 2083 and CSV 23 in grain production.

But in case of dry fodder yield variety CSV 23 provided maximum dry fodder which was statistically at par with genotype SPV 2083 and both these genotypes were obtained analytically higher dry fodder yield over rest of the genotypes tested. The increases with CSV 23 and SPV 2083 were to the tune of 14.67 & 11.38 per cent over CSH 16 and 13.23 & 9.97 per cent over CSH 25, respectively.

In case of biological yield all the genotypes were failed to show any significant response with one another.

The higher grain and fodder yield registered by SPH 1674 and CSV 23 over rest of the genotypes appear to be a resultant of remarkable improvement in different yield components, which was brought about due to adoption of genotypes. It was further, confirmed by the fact that seed yield was found strongly correlated with different yield components. Such close association ship of grain yield with different yield components was also observed by Kumar *et al.* (2008), Sumeriya and Singh (2008), Dhaker (2010) and Mawliya (2012). Chandravanshi, *et al,* (2014) and Thiruna *et al.* (2014)

An examination of data reveals that genotypes SPH 1674 recorded significantly maximum harvest indexover rest of the genotypes. Genotypes SPH 1680,CSH 16 and CSH 25 were proved second best in harvest index and all were significantly superior over SPV 2083 and CSV 23. The increases with genotypes SPH 1674 were by 4.93, 6.55 and 50.57 percent over CSH 16, CSH 25 and CSV 23, respectively.

It can be inferred from the data presented in Table 3 reveals that SPH 1674 recorded significantly maximum gross and net returns as well as B/ C ratio over rest of the genotypes. Genotypes CSH 16, SPH 1680 and CSH 25 were found second best and provided significantly higher gross, net return and B/ C ratio over both the varieties i.e. CSV 23 and SPV

2083 and proved statistically at par with one another in all these parameters. The increases with genotype SPH 1674 were to the magnitude of 8.80, 32.72, 7.06, 7.34 and 31.86 per cent ingross 11.85, 47.69, 9.46, 9.84 and 46.30 per cent in net returns and 12.20, 49.07, 9.15, 9.89 and 47.71 percent in B/ C ratio over SPH 1680, SPV 2083, CSH 16, CSH 25 and CSV 23**,** respectively.

It is obvious because grain yield increased with the increase in the fertility levels in proportion to cost of cultivation hence the gross returns and net returns. Similar observation was also made by Singh and Sumeriya (2006), Dhaker (2010) and Kumawat (2013) on maize and Singh *et al*., 2014 sorghum crop.

Table 1. Response of sorghum genotypes to different fertility levels on nutrient uptake and protein yield

Treatments	Nutrient uptake(kg ha^{-1})									Protein yield (kg ha^{-1})		
	Nitrogen			Phosphorous			Potassium					
	Grain	Fodder	Total	Grain	Fodder	Total	Grain	Fodder	Total	Grain	Fodder	Total
Fertility levels (%RDF)												
0	64.66	45.80	110.46	11.11	16.85	27.96	19.34	157.20	176.54	404.11	286.26	690.37
50	80.90	58.32	139.22	13.72	21.06	34.78	24.17	193.14	217.30	505.64	364.48	870.12
75	92.62	63.04	155.66	16.09	21.91	38.01	28.70	204.64	233.34	578.86	393.99	972.85
100	97.42	69.85	167.27	16.91	23.82	40.72	30.43	229.26	259.69	608.86	436.58	1045.44
SEm±	1.74	1.05	2.04	0.31	0.37	0.50	0.51	3.58	3.61	10.89	6.59	12.78
CD (P=0.05)	4.96	3.00	5.82	0.88	1.06	1.41	1.46	10.20	10.27	31.00	18.75	36.37
Genotypes												
SPH 1674	105.41	58.50	163.92	17.70	20.45	38.14	30.95	193.77	224.71	658.84	365.64	1024.48
SPH 1680	90.55	56.73	147.27	15.77	19.96	35.73	27.74	188.65	216.39	565.91	354.53	920.44
SPV 2083	59.30	62.29	121.59	11.25	21.96	33.22	18.90	202.22	221.12	370.64	389.32	759.96
CSH 16	94.83	56.22	151.04	15.53	19.92	35.44	28.95	189.61	218.56	592.66	351.35	944.01
CSH 25	94.04	57.50	151.55	15.40	20.51	35.91	28.51	192.42	220.93	587.77	359.39	947.16
CSV 23	59.26	64.28	123.54	11.09	22.66	33.76	18.91	209.68	228.60	370.39	401.74	772.13
SEm±	2.13	1.29	2.50	0.38	0.46	0.61	0.63	4.39	4.42	13.34	8.07	15.65
CD (P=0.05)	6.07	3.68	7.13	1.08	1.30	1.73	1.78	12.49	NS	37.97	22.97	44.55

NS= Non significant

Table 2. Response of sorghum genotypes to different Fertility levels on available nutrient status of soil after harvest.

Treatment	N in soil (kg ha^{-1})	P in soil (kg ha^{-1})	K in soil (kg ha^{-1})
Fertility levels (% RDF)			
0	248.69	19.28	338.83
50	257.55	20.17	351.25
75	260.86	20.55	354.14
100	265.18	21.09	367.67
SEm±	1.92	0.23	2.45
CD (P=0.05)	5.46	0.65	6.98
Genotypes			

SPH1674	245.24	19.02	347.65
SPH1680	255.16	19.12	349.49
SPV2083	260.98	20.98	361.57
CSH16	257.03	20.60	350.21
CSH25	260.73	20.56	350.94
CSV23	269.27	21.36	357.97
SEm±	2.35	0.28	3.00
CD (P=0.05)	6.69	0.80	8.55

Table 3. Response of sorghum genotypes to different fertility levels on yield (kg ha^{-1}), harvest index, gross, net returns and B/C ratio.

Treatment	Yield (kg ha^{-1})			Harvest index (%)	Gross returns (` ha^{-1})	Net returns (` ha^{-1})	B/ C Ratio
	Grain	Fodder	Biological				
Fertility levels (% RDF)							
0	4112	10134	14246	28.53	57839	40539	2.34
50	4976	12182	17158	28.88	69859	50824	2.67
75	57.00	12618	18318	31.13	77820	57917	2.91
100	5935	13661	19596	30.53	81893	61122	2.94
SEm±	099	215	236	0.52	1046	1046	0.05
CD (P=0.05)	281	613	672	1.48	2978	2978	0.15
Genotypes							
SPH 1674	6194	11792	17986	34.48	81395	62143	3.22
SPH 1680	5580	11522	17102	32.68	74814	55561	2.87
SPV 2083	4001	12922	16922	23.32	61328	42076	2.16
CSH 16	5688	11602	17291	32.86	76026	56773	2.95
CSH 25	5644	11750	17394	32.36	75827	56574	2.93
CSV 23	3978	13304	17282	22.90	61727	42475	2.18
SEm±	121	264	289	0.64	1281	1281	0.06
CD (P=0.05)	344	751	NS	1.82	3647	3647	0.18

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RESEARCH ARTICLE

ISOLATION AND MOLECULAR CHARACTERIZATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA FROM THE HIGH ALTITUDE HIMALAYAN REGION OF UTTARAKHAND

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Abstract: The objective of this study was to isolate and characterize a rhizospheric bacterium from Munsyari, (2200 feet, 30.06°N/80.23° E) Uttarakhand, western Himalayas, (India). Plant growth promoting rhizobacteria (PGPR) are known to influence plant growth by various direct or indirect mechanisms. Isolated strain was tested for various PGP traits like 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity, phosphate solubilisation, indole acetic acid production, production of siderophore, carbohydrate utilization test. Bio-control ability of isolate was also screened. Further identification of isolate was performed by PCR based 16S rRNA gene sequencing. The isolate PS03 was found to be most effective.

Keywords: Rhizobacteria, Isolation, Molecular, Himalaya region

INTRODUCTION

World population is expected to be 9.7 billion by the year 2050 and to feed this population we need to grow 60% more food (United nation Population Division 2015).This demand can met by increasing the input of chemical fertilizers and increasing the land area under cultivation. But the area under agriculture is already under the urbanization pressure. However, the prices and availability of these chemical fertilizers become the limiting factor for crop production especially in developing countries around the world. Continuous application of Chemical fertilizers may result in negative impacts on agro-ecosystem such as leaching, pollution. Chemical fertilizers pose a detrimental effect on plant, animals and soil health by interference with their natural structure, function and mechanism. In the past few years, researchers all around the world proved the worth and role of plant growth promoting rhizobacteria (PGPR) and mycorrhiza in sustainable, cost effective and nature friendly importance in agriculture. PGPBR may promote plant growth directly usually by either resource facilitation and modulating plant hormone level or indirectly by decreasing the inhibitory effect of various pathogenic agent on plant growth and development, that is, by acting as a biocontrol agent (Glick et al; 1995) Root-colonizing plantbeneficial bacteria, commonly referred to as plant growth-promoting rhizobacteria (PGPR), are capable of stimulating plant growth when cultivated in association with a host plant (Vessey 2003; Hayat et al. 2010). These bacteria are associated with the rhizosphere, the narrow zone of soil surrounding the root that is under the immediate influence of the root system (Dobbelaere et al. 2003) and that provides an important soil ecological environment allowing plant-microbe interactions (Hayat et al. 2010). The rhizosphere provides a rich source of energy and nutrients to the bacteria resulting in higher bacterial diversity and larger populations when compared with bulk soil (Gray and Smith 2005). Likewise, rhizobacteria also secrete a wide variety of metabolites into the rhizosphere that are utilized by plants (Van Loon 2007). Hence it becomes imperative to isolate and characterize new plant growth promoting bacteria and mycorrhiza from soil. Hence this study was aimed to isolate and characterize PGPRs.

MATERIAL AND METHOD

Soil Sample Collection

Rhizospheric soil sample was collected from agricultural soil of Munsyari, (2200 feet, 30.06°N/80.23° E) Uttarakhand, western Himalayas.

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Isolation of Bacteria

Serial dilutions were prepared from the soil samples, and 100 µL aliquots from each dilution of 1 × 10-6, 1 × 10-7, and 1 × 10-8 CFU mL-1 were spread on agar plates and incubated for 24 hours at 25 ± 2°C. Morphologically distinct bacterial colonies were selected for further purifications. The purified isolates were preserved temporarily in 20% glycerol solution at -20°C. Initially 40 strains were selected as potential plant growth promontory bacteria but after different experimentation only five strains were found suitable for further study.

P solubilization Assay

Phosphate solubilization by bacterial isolates was done by the method of Pikovskaya (Pikovskaya et al. 1948) Plates were made in triplicate for each bacterial isolate using Pikovskaya agar medium. Bacterial culture was point inoculated at the center of Pikovskaya agar plate and incubated in incubator at 28°C for 7 day. The plates were then examined for halo zone around bacterial culture and solubilization index (S.I.) was calculated as: S.I. = (colony diameter + halo zone diameter)/colony diameter (Edi-PremonoMoawad et al 1996).

IAA, Siderophore, Carbohydrate utilization and other PGPR activities:

Indole acetic acid (IAA) production by bacterial isolates was determined in LB broth supplemented with L-Tryptophan (500 µg/mL) at 24, 48, and 72 h as described by Patten and Glick (Pattern and Glick et al 2002). For this, bacterial cells were removed by centrifugation at 10,000 rpm for 5 min at 4°C. One mLof the supernatant was mixed with 4 mL of Salkowski's reagent in the ratio of 1: 4 and incubated at room temperature for 20 min. Development of a pink colour indicated indoles. Siderophore production was determined by using blue indicator dye and chrome azurol S agar (Schwyn B, Neilands JB et al 1987). Bacterial isolates exhibiting orange halo zone on chrome azurol S agar after 5 d of incubation at 28°C were considered positive for the production of siderophores.

Antibiotic Sensitivity

Antibiotic sensitivity profile of the strainswas checked by using OD043- 1PK Octadiscs (HIMEDIA, INDIA)

Carbohydrate Utilization Test

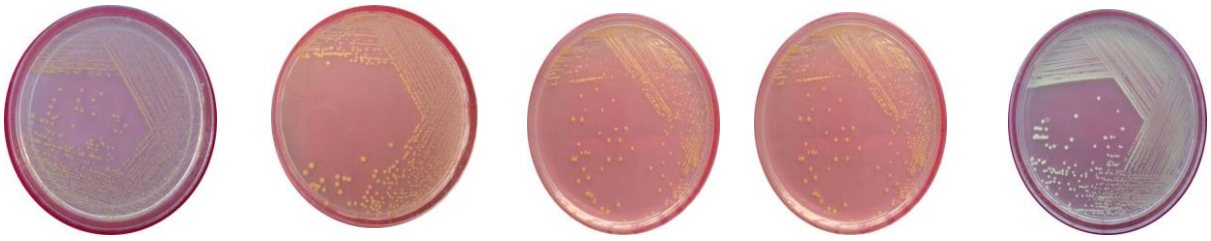
Biochemical characterization was performed using Biochemical test kit i.e. KB009A and KB009B1 (Hi-media, India) according to the manufacturer guidelines. 24h old culture was used for these biochemical tests. 1 mLaliquot of culture was added in the wells and the results were observed after 24h.

Identification of Potent PGPR based on 16S rRNASequencing Genetic characterization based on 16s rRNA gene sequence was analyesd. Genetic DNA was extracted as described by Neumann et al; 1992 and PCR amplification of 16s rDNA was carried out by using primers RDNA 1A 5'AGAGTTTGATCCTGGCTCAG 3' and RDNA 1B 5'AAGGAGGTGATCCAGCCGCA 3' PCR was done as a hot start of 940C for 3minutes followed by 35 cycles of 940C for 1minute, 540C for 1minute, 720c for 1.5 minutes. Amplified PCR products were purified QIAquick gel extraction kit (QIAGEN, GERMANY) and sequenced at automated DNA sequencer (Applied Biosystems 3730). Obtained sequences were compared with Genebank database of NCBI with blastnprogramme and then deposited in Genebank under accession no. KU925853, KU925855 KU925854 KU925856 and KU925857 for PS-01, PS-02, PS-03, PS-04 and PS-05 respectively.

RESULT AND DISCUSSION

Characterization of Bacterial Isolates

All studied bacterial cultures were gram negative except PS02 strain.(Fig.1) Out of five bacterial isolates PS-01 were found to light yellow, PS-03 were found to form yellow brown and rest were showing off-white colour under microscope(Table3.1).Based on 16S rDNA gene sequences, PS-01 and PS-03 isolates were identified as Bacillus species (GenBank accession number KU925853, KU925855 for PS-01 and PS-03 respectively) while the PS-02, PS-04 and PS-05 isolates wereidentified as Microbacterium sp., Pseudomonas sp., and Arthobacter sp. (GenBank accession number KU925854, KU925856 and KU925857 for PS-02, PS-04 and PS-05 respectively).



PS01 PS02 PS03

Fig. 1. Colony characteristics of the bacterial isolates

Phosphate Solubilisation

In the fields, phosphorous is the second most essential macronutrients for the plant growth and development.In the soil, 20–80% of phosphate is in organic form (Richardson et al; 2000) and plant may poorly/not possess an innate ability to acquire phosphorus directly from soil phytate (Greiner et al; 2001). Therefore the availability of P_i is highly dependent on the chemical composition and biological processes occurring in the soil, especially in rhizosphere. Hence these isolated PSB's were analyzed for their P solubilisation activity which degrades the soil phytate to lower phosphate esters which are available to plants. All of the five bacterial cultures showed translucent region around colonies on phytase screening medium described by (Kerovuo et al 1998). The bacterial isolates solubilized tricalcium phosphate in Pikovskaya media. Microbacterium sp. PS-02 showed highest solubilisation index of 4.9, while Bacillus sp. PB-01 showed least solubilisation index 1.39. Bacillus sp. PS-03 and Pseudomonas PS-04 showed the intermediate solubilisation index of 4 (Fig. 3.2, Table 3.2).The results indicate that indigenous bacterial strains could serve as efficient biofertilizer candidates P nutrition of crop plants.

PS01 PS02 PS03 PS04 PS05

Fig. 2. Phosphate solubilization potential on Pikovskaya agar plates of isolates.

Table 1. Growth promotory properties of the bacterial strains

S.No.	Culture ID	Species Name	Phosphate Solubilization (SI)	IAA Production	Siderophore production
1.	PS01	*Bacillus sp strain* 1	+ (1.39)	+ve	-ve
2.	PS02	*Microbacterium sp* strain 2	+ (4.9)	+ve	+ve
3	PS03	*Bacillus sp.* strain 3	+ (4.3)	+ve	-ve
4	PS04	*Pseudomonas sp.* strain 4	+(4.1)	+ve	+ve
5	PS05	*Arthrobacter sp.* strain 5	_	+ve	+ve

IAA, and Siderophore Production

Indole acetic acid (IAA) is one of the most physiologically active auxins. PGPRs enhance the growth of plants by proliferation of lateral roots and root hairs. Rhizospheric bacteria also produce the siderophores which helps the strategy II plants like cereals to uptake the iron and zinc from the soil. All five bacterial cultures were found to be IAA producing, which may enhance plant growth and may help plant to gain good vigour index.PS02, PS04 and PS05 were positive in siderophore production with pink halo zones around colonies (Table 3.2, Fig. 3.3). The bacterial strain PS02 was found to be positive for IAA and siderophore production which is good for the plant productivity.

PS01PS04 PS02 PS03PS05 PS04 PS05

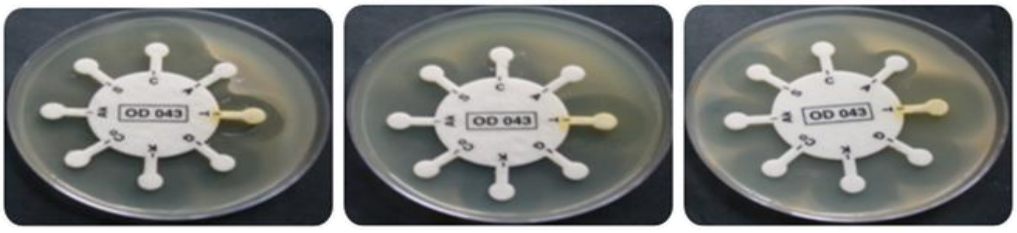
Fig. 3. Siderophore production by potential bacterial strains at 28^{o}C on CAS-blue agar plates

Antibiotic Sensitivity

Plant roots are the main part of the plant for the uptake of minerals and water from the soil. The rhizosphere is the narrow zone of soil specifically influenced by the root system. This zone is rich in nutrients when compared with the bulk soil due to the accumulation of a variety of plant exudates, such as amino acids and sugars, providing a rich source of energy and nutrients for bacteria (Gray and Smith, 2005). Root associated bacteria can be deleterious to the plant. The PGRs indirectly helps the plant by inhibiting the growth of pathogenic bacteria in the rhizosphere(Glick, 1995). Culture PS01, PS02 and PS04 were resistant to Ampicillin but PS03 and PS05 were sensitive to Ampicillin. All remaining bacterial cultures were sensitive to Tetracycline, Gentamycin, kanamycin Co-Trimoxazole, Amicamycin, Streptomycin and Chloramphenicol. (Fig 3.4 & Table 3.4)

Table 2. Antibiotic sensitivity profile of the strains using OD043- 1PK Octadiscs (HIMEDIA, INDIA)

S.N.	Antibiotics	PS01	PS02	PS03	PS04	PS05
1.	**Ampicillin**	-	-	+	-	+
2.	**Tetracycline**	+	+	+	+	+
3.	**Gentamycin**	+	+	+	+	+
4.	**Kanamycin**	+	+	+	+	+
5.	**Co-Trimoxazole**	+	+	+	+	+
6.	**Amikamycin**	+	+	+	+	+
7.	**Streptomycin**	+	+	+	+	+
8.	**Chloremphenicol**	-	+	+	+	+



PS-01 PS-02 PS-03

PS-04 PS-05

Fig .4. Antibiotic Sensitivity profile of strains 1,2,3,4,5

Table 4. Carbohydrate Utilization Test - All the strains were found to be different for utilization of different carbohydrates

Bacteria/ Glucose	PS01	PS02	PS03	PS04	PS05
Lactose	_	_	+	_	_
Xylose	_	+	+	+	+

Maltose	_	+	+	_	+
Fructose	_	+	+	+	+
Dextrose	_	_	+	+	+
Galactose	+	_	+	+	_
Raffinose	_	_	+	_	+
Trehalose	_	_	+	_	_
Mellibiose	_	_	+	_	+
Sucrose	_	+	+	+	+
Arabinose	+	+	+	+	+
Mannose	_	+	+	+	_
Inulin	-	-	+		+
Sodium Gluconate	-	-	-	-	-
Glycerol	-	+	-	+	+
Salicin	-	+	+	+	-
Dulcitol	-	-	-	-	-
Inositol	-	-	-	-	-
Sorbitol	-	-	-	-	-
Mannitol	-	+	-	+	+
Adonitol	-	-	-	-	-
Arabitol	-	-	-	+	+
Erythrytol	-	-	-	-	-
Alpha Methyl D Glucoside	-	+	-	-	-

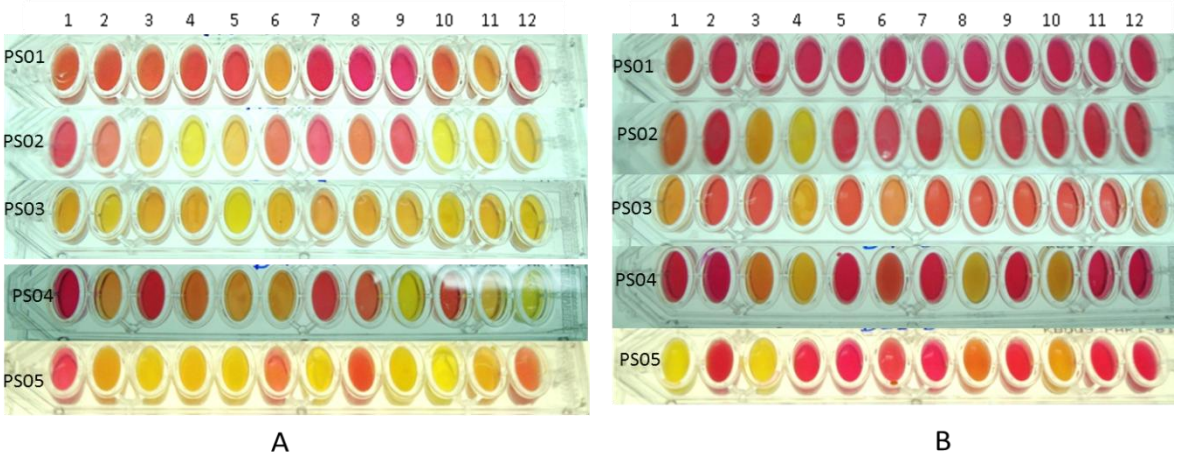


Fig. 4. Carbohydrate utilization tests (Positive-Yellow; Negative-Pink)(A) (1-Lactose,2-Xylose,3-Maltose,4-Fructose, 5-Dextrose, 6-Galactose, 7-Raffinose, 8-Trehalose,9- Melibiose,10- Sucrose,11- L-Arabinose, 12-Mannose), (B) (1-Inulin, 2-Sodium gluconate,3- Glycerol,4- Salicin, 5-Dulcitol,6-Inositol, 7-Sorbitol,8-Mannitol,9- Adonitol,10-Arabitol,11- Erythritol,12- alpha-Methyl-D-glucoside)

CONCLUSION

Biofertilizers is very important for the agriculture as they are cheap, ecofriendly and efficient, hence important for the sustainable agriculture. Hence, plant growth promoting rhizobacteria (PGPR) isolation and characterization is very important for utilizing them as Biofertilizers. If a PGPR is isolated from the harsh environment like high altitude it has the added advantage of to withstand the abiotic stress. In our study we have isolated the different bacterial strains from high altitude. All the isolates gram negative except the one. Two isolates were identified as Bacillus species (PS-01 and PS-03) rest three (PS-02, PS-04 and PS-05) were identified as Microbacterium sp., Pseudomonas sp., and Arthobacter sp. Isolate PS03 was found to be most efficient for Phosphate utilization, siderophore production and carbohydrate utilization and

antibiotic profiling. Hence this isolate PS-03 should prove a better PGPR.

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RESEARCH ARTICLE

SURVEY FOR INCIDENCE, SEVERITY AND SCREENING OF BRINJAL GERMPLASM LINES AGAINST FRUIT ROT DISEASE OF BRINJAL

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Abstract: A survey was conducted during September to November, 2014 to observe disease prevalence of brinjal fruit rot in Bagalkot district at Northern dry zone of Karnataka. Through the survey disease severity and incidence were recorded. The roving survey revealed the presence of disease in all talukas *viz.*, Bagalkot, Badami, Hunagunda, Jamakandi and Mudhol. The per cent disease index ranged from 13.00 to 54.66. Per cent disease index was high in Bagalkot taluk followed by Badami and Jamakandi taluk. Among different villages under cultivation in these districts, Belur was more prone to disease with per cent disease index of 54.66 followed by Sulikieri which recorded a per cent disease index of 44.00. Screening of 60 genotypes under field conditions revealed that none of the genotypes were found to be immune. Only two genotypes were found resistant and 31 genotypes showed moderately resistant reaction and 27 genotypes showed moderately susceptible reaction.

Keywords: Egg Plant, Pathogenic Fungi, *Solanum melongena*. L., Brinjal

INTRODUCTION

Brinjal (*Solanum melongena* L.) is one of the most important vegetables in South Asia which accounts for almost fifty percent of the world area under cultivation and also popular in some parts of Africa and Central America (Harish *et al.,* 2011). In India, brinjal is an important and indigenous vegetable crop often known as the cash crop for the farmers. Its centre of origin is in the Indo-Burma region (Vavilov, 1928), In India, brinjal is mainly grown in the states like West Bengal, Orissa, Bihar, Gujarat, Maharastra, Andhra Pradesh, Karnataka etc. with an area of 7.22 lakh hectare with a production of 135.58 metric tonnes and productivity of 19.10 tonnes per ha (Anon., 2014). It contributes about 12.47 per cent of the total production of vegetables in India. In Karnataka, brinjal is cultivated over an area of 15,800 ha with a production of 4002.50 tonnes (Anon., 2014). It is mainly grown in the Bagalkot district. The immature tender fruits are used as vegetable, pickle making and in dehydration industries. It can also cure tooth ache, brinjal fruit cooked in til oil acts as an excellent remedy for those suffering from liver complaints (Chauhan, 1981). Contrary to common belief, it is quite high in nutritive value and every 100g of edible portion contains 92.7g moisture, 6.4g carbohydrate, 1.3g protein, 0.3g fat, 1.3g fiber, 124 IU vitamin A, 0.09 mg nicotinic acid, 120 mg vitamin C, 200 mg potassium, 18 mg calcium, 16 mg magnesium, 47 mg phosphorus and 0.9 mg iron (Aykroyd, 1963). There are some biotic and a biotic stresses which are limiting the successful production of brinjal. Among the biotic stresses, the fruit rot complex caused by many fungi is one of the threatening diseases. Though, it is suspected that many fungi are involved, the exact role of these fungi is not documented. And some farmers are using known fungicides indiscriminately and unscientifically which may result in residual toxicity problems in brinjal fruit. On the other hand no resistant variety / line/ germplasm are available for this disease. Hence there is alternative look for the assessment of genetic variation, is a major concern of plant pathologists, breeders and population geneticists. Availability of sufficient variation is required for the production of new varieties that are aimed towards the improvement of crop productivity and able to withstand damage from biotic and a biotic factor. Hence there is need to determine the resistance source.

The literature reveals that not much work has been done on these aspects. Therefore, the following stydy were undertaken in the present study for the management of the disease and survey for collection and assessment of brinjal fruit rot disease severity and screening of genotypes against fruit rot of brinjal and identification of resistance source in Bagalkot district.

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MATERIAL AND METHOD

The survey was carried out during September to November, 2014 to observe the prevalence of fruit rot in brinjal. Survey was carried out in 5 taluks of Bagalkot district *viz.*, Badami, Bagalkot, Hunagunda, Jamakandi and Mudhol. In each taluk five villages were selected and from each village two farmers' fields were selected for recording observations. The incidence and severity of disease were recorded by visual observation in three different spots in a single field. In each spot, 30 fruit rot samples were evaluated for the disease incidence and severity. The severity in terms of per cent Disease Index (PDI) was recorded on fruits by grading them using the 0-5 scale as given by Islam *et al.*,1990 (table 1). The per cent fruit infection and per cent disease index were calculated by formula given below.

$$\text{Per cent fruit infection} = \frac{\text{No. of fruits infected}}{\text{Total no of fruits counted}} \times 100$$

Table 1. Scale for scoring the fruit rot of brinjal (Islam *et al.*, 1990)

Sl. No	Grade	Description
1	0	0% infection on fruit
2	1	1-10% infection on fruit
3	2	10-15% infection on fruit
4	3	15-30% infection on fruit
5	4	30-40% infection on fruit
6	5	50% infection on fruit

The experiment on screening of 60 brinjal lines against fruit rot was conducted at Haveli farm of College of Horticulture Bagalkot, Karnataka, under the natural infection. Seedlings were raised in plastic trays in the net house with proper care and management. A piece of medium high land with good drainage system was selected. The field was prepared by ploughing and harrowing. During field preparation, fertilizers and manures were applied at recommended doses (Anon, 1997). Seedlings of 30 days old were transplanted in the field and watered properly. The lines were planted in the 6m single line in two replications along with the available susceptible line (line no-26) in between every 5 lines. Five seedlings of each line were planted at 60×60 cm spacing and each line was replicated twice. Observations were recorded by screening the lines under natural disease pressure conditions. The lines were graded according to the 0 to 5 scales as suggested by (Islam *et al.*, 1990) and finally PDI was calculated. Sixty genotypes/lines were evaluated under field condition to know their disease reaction against fruit rot of brinjal. Per cent disease index was calculated as described in Table 2. Further the varieties were placed in different categories of resistance and susceptibility on the basis of method given by Pathak *et al.* (1986).

Isolation

Rotten fruits were collected from plantations for investigation in the laboratory. The pathogens were isolated by cutting discs (3mm thick) of rotten tissue under aseptic conditions, after surface sterilization in 0.01% mercuric chloride. The discs were then plated on PDA and other media incubated at 30^0C. Pure isolates of the causative organisms were obtained by the standard technique. 5 samples from each taluka were brought to lab and sorted. For identification of diseases the temporary and permanent slides of the pathogens were prepared in lab. The authenticity of pathogen was established through Koch's postulates. The pathogens were also cultured in lab and were kept in the form of permanent pure culture for further physiological and cultural studies Different media viz. PDA, oat meal agar, corn meal agar, Richard's agar, Asthana and Hawker's and malt extract agar were utilized.

Proving pathogenecity

To confirm the identity of isolated pathogens, pathogenecity tests were performed with fruits of same age of highly susceptible variety (Line -26). Apparently healthy fruits were taken from plants, washed with sterilized distilled water and placed in desiccators. Spore suspension was made from 7 days old cultures with sterilized distilled water and diluted to 250-500 spores per microscopic field. Fruits were inoculated with spore suspension of different pathogens by pin prick method and incubated at 25±2°C temperature and R.H >90% and examined regularly for appearance of characteristic symptoms of disease. The part of the fruit showing characteristic symptom was taken and the pathogen was re isolated by following standard tissue isolation method. The re isolated cultures were again compared with original culture for morphological and cultural characters with the original culture. The temporary and permanent slides of the pathogens were prepared in laboratory. The pathogens were kept in the form of permanent pure culture for further physiological and cultural studies.

RESULT AND DISCUSSION

A roving survey to know the incidence of fruit rot of brinjal was carried out in five taluks of Bagalkot

district *viz.*, Badami, Bagalkot, Mudhol, Jamakandi and Hunagunda. Five villages from each taluk and two fields in each village were surveyed. Incidence of fruit rot of brinjal was noticed in all the places surveyed on two commonly grown local brinjal cultivars and Mahycho super-10 hybrids. The results are described as in Table 1. In Bagalkot taluk the per cent disease index (PDI) ranged from 21.33 to 54.66. The highest PDI of 54.66 was recorded at Belur followed by Anadinni (36.00) village and Jalyal recorded least (21.33). In Bagalkot taluk highest fruit rot infection of 86.67 was recorded in Belur followed by 46.66 in Anadinni and 40.00 in Irapur. Sannadinni and Jalyal (33.33) recorded least per cent fruit infecton among all villages.In Badami per cent disease index (PDI) ranged from 17.33 to 44.00.The highest PDI was recorded in Sulikeri (44.00) followed by Asangi (41.33) and Holealur (38.66) and Badagi (37.33). The lowest PDI was recorded in Kerkalmatti (17.33). The highest per cent fruit rot infection was recorded in Asangi (66.67) and Sulikeri (66.67) and Badagi (37.33) followed by Holealur (38.66). Least per cent fruit rot was observed in Kerakalmatti (46.67). In Jamakandi taluk Tummankatti (29.33) recorded highest per cent disease index followed by Navalagi (25.00) and Mahalingapur (25.00). The least incidence was recorded in Dawaleswar (13.33) and Jagadhal (13.33). The data pertaining to fruit infection reveals that Tummanakatti (53.33) recorded highest followed by Navalagi. The lowest fruit infection was recorded in Dawaleswar (20.00) among the five villages surveyed. In Mudhol taluk highest per cent disease incidence was recorded in Mugalkod (41.33) followed by Hebbal (35.00), Belagali (33.33) and Muddapur (26.66). The lowest was recorded in Kadakol (21.66). The data recorded with respect to the fruit rot infection resulted that Mugalkod (80.00) recorded highest fruit rot infection followed by Belagali (53.33) and Hebbal (53.33). The least per cent of fruit rot infection was recorded in Muddapur (46.67). In Hunagunda taluk Kamatagi recorded highest per cent disease incidence followed by Rakkasagi (29.00) and Aminagada (25.00). The least incidence was recorded in the village Kamblihal (13.00) followed by Gorbal (17.00). The data recorded with respect to the fruit rot infection revealed that Rakkasagi (53.00) recorded highest followed by Gorbal (46.67) and Kamatagi (40.00). The least fruit rot infection was recorded in the village Kamblihal (20.00). A detailed survey was undertaken in few parts northern Karnataka to gather information on the incidence and spread of *Alternaria alternata*, *Colletotrichum melongenae* and *Phomopsis vexans* causing fruit rot of brinjal from different localities of Bagalkot district. This information is highly useful to identify the hot spots for this disease in Bagalkot district where brinjal is extensively grown as commercial crop. From the survey it is evident that the incidence of this disease varied from locality to locality depending on the type of variety cultivated and management practices followed. The incidence of the disease was also dependent on inoculum load and environmental conditions prevailing in different localities. Among different the taluks surveyed the highest per cent disease index (54.66) of fruit rot was noticed in Belur village of Bagalkot taluka and the lowest (13.00) in Kamblihal village in Hunagunda taluka indicating that the disease was not consistent in all localities. These results are close conformity of Hossain *et al.* (2010) who conducted the survey on major diseases of vegetable and fruit crops including fruit rot of brinjal in Chittagong region and found that the amount of crop and fruit losses to particular disease varied from place to place because of the existence of different races, biotypes or strains of the pathogen. Sharma *et al*. (2011) conducted extensive periodic survey of major brinjal growing areas of Jammu division. The survey revealed presence of the disease in all the locations with varying per cent incidence and intensity. The fruit rot of brinjal was severe in Bagalkot taluk than in other taluks. This could be because of favourable environmental conditions and initial inoculum prevailed. Also could be continuous growing of the crop. The variety/hybrid used cultivation practices and disease management practices in Bagalkot (not practicing crop rotation and management practices as evidenced during survey) taluk vary with other taluk. This might have helped in rapid development of the disease in further stages of the crop growth when environmental conditions became congenial. Sixty brinjal genotypes were screened against fruit rot under natural epiphytotic condition as described in material and methods and the results are presented in Table 2. The data revealed that, among the 60 genotypes none of them were found immune. Per cent Disease Index ranged between (15.00 –40.40%). Two genotypes *viz.*, CBB-3 (10.50) and CBB-26 (15.52) were found resistant, 31 genotypes *viz.*, CBB-1 (18.84), CO-2 (22.45), CBB-5 (16.20), CBB-6 (24.60), CBB-7 (20.10), CBB-11 (23.10), CBB-15 (21.26), CBB-16 (18.00), CBB-17 (18.45), CBB-19 (18.64), CBB-20 (20.60), CBB-22 (25.40), CBB-27 (25.36), CBB-28 (21.28), CBB-30 (20.46), CBB-31 (24.62), CBB-32 (25.34), CBB-33 (16.26), CBB-34 (30.04), CBB-37 (18.60), CBB-41 (22.42), CBB-43 (16.84), CBB-44 (20.62), CBB-45 (25.46), CBB-46 (21.80), CBB-50 (24.32), CBB-54 (25.50), CBB-56 (20.45), CBB-57 (24.64), CBB-58 (20.20) and CBB-59 (16.40) showed moderately resistant reaction, 27 genotypes *viz.*,CBB-2 (30.20), CBB-4 (27.00), CBB-8 (30.25), CBB-9 (28.30), CBB-10 (35.20), CBB-12 (36.00), CBB-13 (27.62), CBB-14 (40.24), CBB-21 (30.00), CBB-23 (32.30), CBB-24 (27.54), CBB-25 (35.40), CBB-29 (30.40), CBB-34 (30.04), CBB-35 (28.43), CBB-36 (26.14), CBB-38 (35.58), CBB-39 (40.40), CBB-40 (32.64), CBB-42 (30.49), CBB-47 (26.74), CBB-48 (28.23), CBB-49 (34.22), CBB-51 (28.20),

CBB-52 (35.56), CBB-53 (36.40) and CBB-55 (27.32) were susceptible. None of the genotypes showed highly susceptible reaction. Breeding for the disease resistance has been an effective, economical and practical method of disease control. Cultivation of resistant variety seems to be the best alternative and most economical to keep the activity of fruit rot pathogen under control. In all crop improvement programmes, growing of resistant varieties has been found to be appropriate choice to combat the disease. The use of resistant cultivars is perhaps the most desirable method of controlling diseases in crops (Wharton and Diéguez-Uribeondo, 2004; Than *et al.*, 2008). This approach, according to Voorrips *et al.* (2004), has been less exploited in fruit and vegetable crops mainly due to the longer time required for breeding and selecting for resistance and the short term advantage of chemical control. Efforts have been made to locate the source of resistance for this disease in India.

In the present investigation, the reaction of different genotypes against fruit rot was carried out in field conditions. Sixty brinjal genotypes were screened against brinjal fruit rot under natural condition as described in material and methods. The data revealed that, among the 60 genotypes evaluated, none was found immune. Two genotypes *viz.*, CBB-3 and CBB-26 were found resistant, 31 genotypes were moderately resistant and 27 genotypes showed susceptible reaction. None of the genotypes showed highly susceptible reaction. The results are in contrary with findings of Pandey *et al.* (2002) who conducted the experiment to evaluate 41 entries of brinjal under natural epiphytotic condition against *Phomopsis* blight disease. Among 41 lines evaluated, none of the entries were found resistant to fruit rot. Two varieties *viz.,* Ramanagar giant and KS-233 showed moderate resistance and others showed susceptibility. However both DBR-91 and baramasi recorded high susceptibility with fruit rot intensity of 4.72 / plant and per cent fruit infection of 47.5% and 85% respectively. In the present investigation, according to phenotypic analysis CBB-1 & CBB-26 were found resistant so, by above result it revealed that same can be used in the breeding strategies for the crop improvement programme to develop resistant varieties.

SUMMARY AND CONCLUSION

Survey revealed the presence of disease in all talukas *viz.*, Bagalkot, Badami, Hunagunda, Jamakandi and Mudhol. The per cent disease index ranged from 13.00 to 54.66. Per cent disease index was high in Bagalkot taluk followed by Badami and Jamakandi taluk. Among different villages under cultivation in these districts, Belur was more prone to disease with per cent disease index of 54.66 followed by Sulkieri which recorded a per cent disease index of 44.00.The isolated fungus *Alternaria alternata, Colletotrichum melongenae and Phomopsis vexans* proved to be pathogenic on brinjal fruits after artificial inoculation (as per pathogenicity). Screening of 60 genotypes under field conditions revealed that none of the genotypes were found to be immune. Only two genotypes were found resistant and 31 genotypes showed moderately resistant reaction and 27 genotypes showed moderately susceptible reaction.

Table 2. Survey for severity of fruit rot of brinjal in Bagalkot district

Taluka	Village	Total Area Surveyed (Acre)	Variety/Hybrid	Percent fruit infection	Percent disease index
Bagalkot	Anadinni	4.5	Mahyco	46.66	36.00
	Belur		Local	86.67	54.66
	Irappur		Mahyco	40.00	32.00
	Jalyal		Local	33.33	21.33
	Sannadinni		Mahyco	33.33	26.62
Badami	Asangi	5.15	Local	66.67	41.33
	Badagi		Local	53.33	37.33
	Holealur		Local	66.67	38.66
	Kerkalmatti		Mahyco	46.67	17.33
	Sulikeri		Mahyco	66.67	44.00
Hunagunda	Amingada	1.25	Mahyco	33.33	25.00
	Gorabal		Local	46.67	17.00
	Kamatagi		Mahyco	40.00	32.00
	Kamblihal		Mahyco	20.00	13.00
	Rakkasagi		Local	53.00	29.00
Jamakandi	Dawaleswar	1.15	Mahyco	20.00	13.33
	Jagadhal		Local	20.00	13.33
	Mahalingpur		Local	33.33	25.00
	Navalagi		Local	40.00	25.00
	Tummanakatti		Local	53.33	29.33

Mudhol	Belagali	1.30	Mahyco	66.67	33.33
	Hebbal		Mahyco	53.33	35.00
	Kadakol		Local	53.33	21.66
	Muddapur		Mahyco	46.67	26.66
	Mugalkod		Mahyco	80.00	41.33

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RESEARCH ARTICLE

AVAILABLE MICRONUTRIENTS IN SOILS OF CHIKKARSINKERE HOBLI OF MADDUR TALUK, MANDYA DISTRICT OF KARNATAKA

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Abstract: Available micronutrients and their relationship with different soil properties was studied in four hundred soil samples collected from different locations of 42 villages representing the soils of Chikkarsinkere hobli of Maddur Taluk Mandya district of Karnataka. The soils were analysed for textural separates, physico-chemical properties and status of available micronutrients. On the basis of pH and EC values, these soils are moderately acidic to very strongly alkaline (5.6 to 9.4). Majority of the soils under study area were found deficient in available zinc. Available iron, copper and manganese were sufficient to adequate. The availability of micronutrients in soils significantly influenced by soil properties viz, textural separates, organic carbon, $CaCO_3$, CEC and pH of soils. Available Zn ranged between 0.02 to 6.36 mg kg^{-1} with a mean value of 0.63 mg kg^{-1}, available Fe ranged ranged from 0.14 to 95.4 mg kg^{-1} with a mean value of 25.29 mg kg^{-1}. Available Cu ranged between 0.14 to 6.10 mg kg^{-1} with a mean value of 1.29 mg kg^{-1}. Available Mn ranged between 1.20 to 40.20 mg kg^{-1} with a mean value of 13.41 mg kg^{-1}. Organic carbon, clay, and CEC were positively correlated with available Zn, Fe, Cu and Mn while pH, $CaCO_3$ and sand were negatively correlated.

Keywords: Available micronutrients, Fertility, Correlation, Critical limit

INTRODUCTION

The productivity of soil mainly depends upon its ability to supply nutrients to the growing plants. The crop productivity can be increased by utilizing the available basic information related to soil analysis, use of agro-ecosystem and management practices of soil. The optimum plant growth and crop yield depends not only on the total amounts present in the soil at a particular time but also on their availability which in turn is controlled by physical and chemical properties of soil. The physico-chemical properties of the soil like mechanical composition, pH, EC, OC and $CaCO_3$ etc. influence the availability of micronutrients which in turn affect the crop yield. Among the micronutrients the deficiency of zinc is wide spread in Indian soils followed by boron in eastern part of country. Calcareous and saline sodic soils are mostly found deficient in iron, manganese and copper. Sakal and Singh (2001) reported that 47 per cent Indian soils were deficient in available zinc. The optimum plant growth and crop yield depends not only on the total amount of nutrients present in the soil at a particular time but also on their availability.

Micronutrients are important for maintaining soil health and also increasing productivity of crops (Ratan *et al*. 2009). The soil must supply micronutrients for desired growth of plants. Increased removal of micronutrients as a consequence of adoption of high yielding varieties (HYVs) and intensive cropping together with shift towards high analysis NPK fertilizers has caused decline in the level of micronutrients in the soil. The improper nutrient management has led to emergence of multinutrient deficiencies in the Indian soils (Sharma 2008). Keeping in view the close relationship between soil properties and available zinc and iron, the present study was undertaken to analysis the influence of soil properties on the availability of zinc and iron for better land use management of soils of Chikkarsinkere Hobli of Maddur Taluk, Mandya district of Karnataka as information available on these soil is rather scanty.

MATERIAL AND METHOD

Location and extent: Chikarsinekere Hobli is situated in Maddur taluk of Mandya district in Karnataka State (Fig. 1), which falls in the southern dry zone. It lies between 76° 58′ to 77° 05′E longitude and 12° 26′ to 12° 34′ N Latitude, and covered by 57 D/14, 57 D/15, 57 H/2 & 57 H/3 Survey of India toposheets. Total area of the hobli is 16,873 ha. Major part of the area (7,478) is under canal irrigation and rainfed area occupies about 4,367 ha. Major part of the Hobli is under Cauvery canal irrigation.

Climate: The Chikkarasinakere hobli enjoys sub-tropical monsoonic climate. The average temperature of the area ranges between 16^0 and 35^0 C. The normal rainfall received in the area is about 770 mm. Out of this, about 50 per cent is received during southwest monsoon, 20 per cent during northeast monsoon and 30 per cent during the summer period.

Geology: Granites and gneiss, commonly known as peninsular gnesis, are the major rock types of the

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hobli. They are the oldest rock formations of the world and belong to the Archean period.

Landforms: The Chikkarsinkere hobli forms part of Bangalore plateau. The elevation ranges from 600 m near Shimsha River to 769 m. The major landforms identified in the area are uplands, lowlands and valleys. The uplands are characterized by nearly level to very gently sloping summits, followed by gently to very gently sloping side slopes, which merges with nearly level lowlands/valleys. The uplands occupy about 55 per cent of the area in the hobli. The lowlands occur mostly below the tanks or between the uplands and valleys. They have flat topography and mostly cultivated to paddy or sugarcane. The valleys and lowlands together occupy about 29 per cent of the area in the hobli.

Natural vegetation: Chikkarsinkere hobli has a number of tree species, shrubs and herbs. The natural vegetation of the area consists of tropical dry deciduos types. Tamarind, Ficus, Mango, Uluchemira, Babul, Acacia, Bage etc. are dominantly found in the area.

Present land use: Out of the total area of 16,873 ha, about 12,739 ha area is under cultivation, which is more than 75 per cent of the total area available in the hobli. Major part of the cultivable lands (7478 ha) are under canal irrigation. The canal irrigated lands are used for the cultivation of rice, sugarcane, coconut, mulberry etc, and rainfed areas are used for the cultivation of ragi, pulses and oilseeds. Vegetables crops like tomato, brinjal, chillies and cucumber are grown in a small area, mostly for local consumption in the area.

Soil sampling: Four hundred representative composite soil samples from a depth of 0-15 cm were collected with the help of a wooden *Khurpi*. Samples were completely air-dried and passed through 2 mm sieve and stored in properly labeled plastic bags for analysis.

Soil analysis: Soil pH was measured in 1:2.5 soil water suspension using glass electrode pH meter. Electrical conductivity was measured in 1:2.5 soil water supernatant solution with the help of conductivity bridge (Jackson 1973). The organic carbon was determined by rapid titration method (Walkley and Black 1934) and $CaCO_3$ by rapid titration method (Puri 1930). The available micronutrients in soil samples were extracted with DTPA (0.005 M DTPA + 0.01 M $CaCl_2$ + 0.1 M TEA, pH 7.3) as per the method described by Lindsay and Norvell (1978) and the concentration of Zn, Fe, Cu and Mn in the DTPA-extract was determined using atomic absorption spectrophotometer.

RESULT AND DISCUSSION

Physico-chemical Properties

The data presented (Table 1) on soil properties showed that the sand content ranged between 25.5 to 89.7 per cent with a mean value of 56.9 per cent, silt content varied from 1.1 to 21.6 per cent with a mean value of 8.1 per cent and clay content varied from 8.1 to 59.9 per cent with a mean value of 35.1 per cent. The soils are moderately acidic to very strongly alkaline (5.6 to 9.4). The alkaline nature of soil under study is attributed to the fairly optimum base saturation in the region (Sharma *et al.* 1992). The electrical conductivity (EC) ranged from 0.02 to 0.62 dSm^{-1} with a mean value of 0.17 dSm^{-1}. All of the soil samples are under < 1 dSm^{-1}. It indicates that they are non saline in nature as suggested by Muhr *et al.* (1963) comparatively low content of soluble salts appear to be due to the type of climate of the area which is fairly sufficient to leach out major part of soluble salts from the soil. The organic carbon ranged from 0.07 to 13.7 g kg^{-1} soil with a mean value of 3.17 g kg^{-1} soil. It showed a considerable variation with types and topography of soil. Relatively higher values of organic carbon can be ascribed to annual addition of plant residues and also the application of FYM (Ashok, 1998). The $CaCO_3$ ranged from 0 to 50.0 g kg^{-1} with a mean value of 18.5 g kg^{-1} is a useful parameter to assess the extent of nutrient availability and their release behaviour. The CEC values ranged from 0.57 to 57.4 cmol (p^+) kg^{-1} with a mean value of 16.17 cmol (p^+) kg^{-1}.

Available Zinc

DTPA-zinc ranged between 0.02 to 6.36 mg kg^{-1} with a mean value of 0.63 mg kg^{-1}. It was observed that zinc was most deficient in Chikkarsinkere hobli (Fig. 2). The DTPA-Zn consistently decreased with increasing depth of soil profile (Naik 2014). Clay and silt are the most active fractions of soil. Most of the zinc bearing minerals such as biotite, hornblende, augite and others are easily weathered and thus released zinc is subjected to secondary soil forming processes such as adsorption of Zn^{2+} ions by clay. Zinc (Zn^{2+}) ions adsorbed on soil complexes may easily be removed by leaching especially in sandy loam soils and adsorbed zinc is in equilibrium with the soil solution zinc. The amount of extracted zinc is likely to increase with the increase in fineness of the soil texture. It has also been reported that organic matter plays an important role in controlling availability of zinc particularly in alkaline soils (Das, 2000). A close examination of the data in Table 2 indicates significant increase in zinc content with increase in organic carbon (r = 0.360*). The availability of zinc increased significantly with increase in organic carbon because zinc forms soluble complexes (Chelates) with soil organic matter component. On the other hand, the availability of zinc reduced significantly with an increase in $CaCO_3$ (r = - 0.201**) and pH (r = - 0.347*) of soil. At high pH and $CaCO_3$ content, zinc forms insoluble compounds such as $Zn(OH)_2$ and $ZnCO_3$ which can reduce the availability of zinc. There was inverse relationship between zinc and pH as the pH increases the availability of zinc decreased. The findings of the

present investigation are confirmed by the results of Singh (2006) and Mehra (2007).

Available Iron

The data presented in Table 1 showed that DTPA-iron ranged from 0.14 to 95.4 mg kg^{-1} with a mean value of 25.29 mg kg^{-1}. The available iron significantly increased with increase in clay (r = 0.375*), organic carbon (r = 0.344*), and CEC (r = 0.439*). On the other hand the availability of iron was reduced significantly with an increase in $CaCO_3$ (r = -0.378*) and pH (r = -0.414*). Further, the availability of iron was non-significantly affected by the other characteristics of soils. It was significantly increased with increase in finer fractions (silt and clay) because these fractions are helpful improving the soil structure and aeration of soils. The available iron was found to increase with increase in CEC of soils due to more availability of exchange sites on soil colloids. The availability of iron enhanced significantly with increase in organic matter because (i) organic matter is helpful in improving soil structure and aeration conditions, (ii) organic matter protect the oxidation and precipitation of iron into unavailable forms and (iii) supply of chelating agents, which increase the solubility of iron compounds. On the other hands, its availability was found to be reduced with increase in pH_2 and $CaCO_3$ contents of soils. Most readily available form of iron is Fe^{2+} ions, which convert into less soluble form (Fe^{3+} ions) after oxidation. High pH is responsible for its oxidation. Hence, the availability of iron reduced at higher pH level. Beside this, at high pH iron is also precipitated as insoluble $Fe(OH)_3$ which reduces its availability. The $CaCO_3$ present in soils gets converted into bicarbonates ions which reduces the availability of iron and the chlorosis caused in these conditions is known as a "lime induced chlorosis". The availability of iron at high pH is reduced due to the reduction in its solubility. The solubility of iron decreased with increase in pH is due to the formation of insoluble iron hydroxide and carbonates. Similar results were reported by Gupta (2003) and Yadav and Meena (2009).

Available copper

The data presented in Table 1 showed that DTPA-copper varied from 0.14 to 6.10 mg kg^{-1} with a mean value of 1.29 mg kg^{-1}. As given in Table 2 the available copper significantly increased with increase in clay (r = 0.421*) and organic carbon (r = 0.252**). On the other hand the availability of copper was reduced significantly with an increase in $CaCO_3$ (r = -0.275*) and pH (r = -0.204**). The organic acid molecules present in organic matter solubilise Cu^{2+} ions by chelation and complexion and as a result of this organic binding, there is more dissolved copper in the soil solution than normally occurs in the absence of organic matter. Furthermore the availability of copper enhanced with increase in silt and clay contents and this might be due to the improvement of soil structure and aeration conditions of soils with increase in finer fractions in soil mass. The availability of copper suppresses significantly with sand contents because the coarseness of soil texture reduces the adsorption of Cu^{2+} ions on exchange sites. The availability of copper reduces at high pH and high $CaCO_3$ content due to the formation of less soluble compounds like $Cu(OH)_2$ and $CuCO_3$. Similar results were reported by Singh *et al.*, (2013).

Available Manganese

The data presented in Table.1 showed that DTPA-manganese varied from 1.20 to 40.20 mg kg^{-1} with a mean value of 13.41 mg kg^{-1}. A close examination of data in Table 2 indicates that the availability of manganese in these soils enhanced with increase in clay (r = 0.593*), organic carbon (r = 0.228*), and CEC (r = 0.194). There was a positive correlation between manganese and organic carbon as the organic carbon content increases the availability of manganese increases. The increase in availability of manganese with increase in clay and silt might be due to the improvement in soil structure and aeration conditions. On the other hand the availability of manganese was reduced significantly with an increase in $CaCO_3$ (r = -0.313*), sand (r = -0.121) and pH (r = -0.233**). The availability of Mn decrease with increase in $CaCO_3$ content and pH of soils might due to the formation of less soluble compounds like $MnCO_3$ or $Mn(OH)_2$. The higher pH favours the formation of less soluble organic complexes of Mn, which reduces the availability of Mn and the activity of soil micro-organism which oxidizes soluble Mn^{2+} (Singh *et al.*, 2013).

Table 1. Ranges and mean values of physico-chemical properties of soils of Chikkarsinkere Hobli.

Ranges	Soil properties											
	Sand (%)	Silt (%)	Clay (%)	pH	EC (dSm^{-1})	OC g kg^{-1}	$CaCO_3$ g kg^{-1}	CEC cmol (p+) kg^{-1}	Micronutrients			
									Zn	Fe	Cu	Mn
									mg kg^{-1}			
Maximum	89.7	21.6	59.9	9.4	0.62	13.7	50.0	57.4	6.36	95.4	6.10	40.20
Minimum	25.5	1.1	8.1	5.6	0.02	0.07	0.0	0.57	0.02	0.14	0.14	1.20
Mean	56.9	8.1	35.1	7.9	0.17	3.17	18.5	16.26	0.63	25.29	1.29	13.41

Table 2. Correlations between soil properties and available micronutrients of soils of Chikkarsinkere Hobli.

Micronutrients	Soil properties							
	Sand (%)	Silt (%)	Clay (%)	pH	EC (dSm^{-1})	OC g kg^{-1}	$CaCO_3$ g kg^{-1}	CEC cmol (p+) kg^{-1}
Zn	-0.258*	0.191	0.413*	-0.347*	0.413*	0.360*	-0.201**	0.265*
Fe	-0.131	0.251**	0.375*	-0.414*	0.168	0.344*	-0.378*	0.439*
Cu	-0.289*	0.316*	0.421*	-0.204**	0.042	0.252**	-0.275*	0.226**
Mn	-0.121	0.136	0.593*	-0.233**	0.148	0.228**	-0.313*	0.194

Level of significance at .05% (**) and .01 % (*)

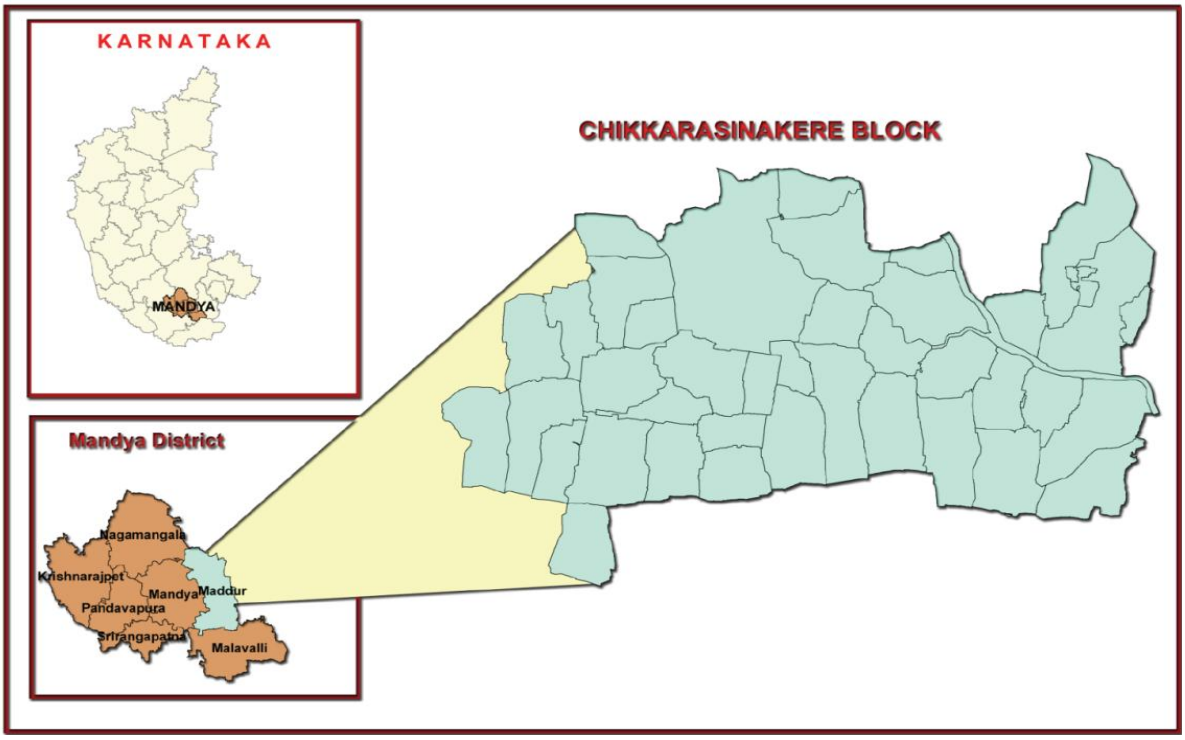

Fig. 1. Location map of Chikkarsinakere Hobli in Mandya district

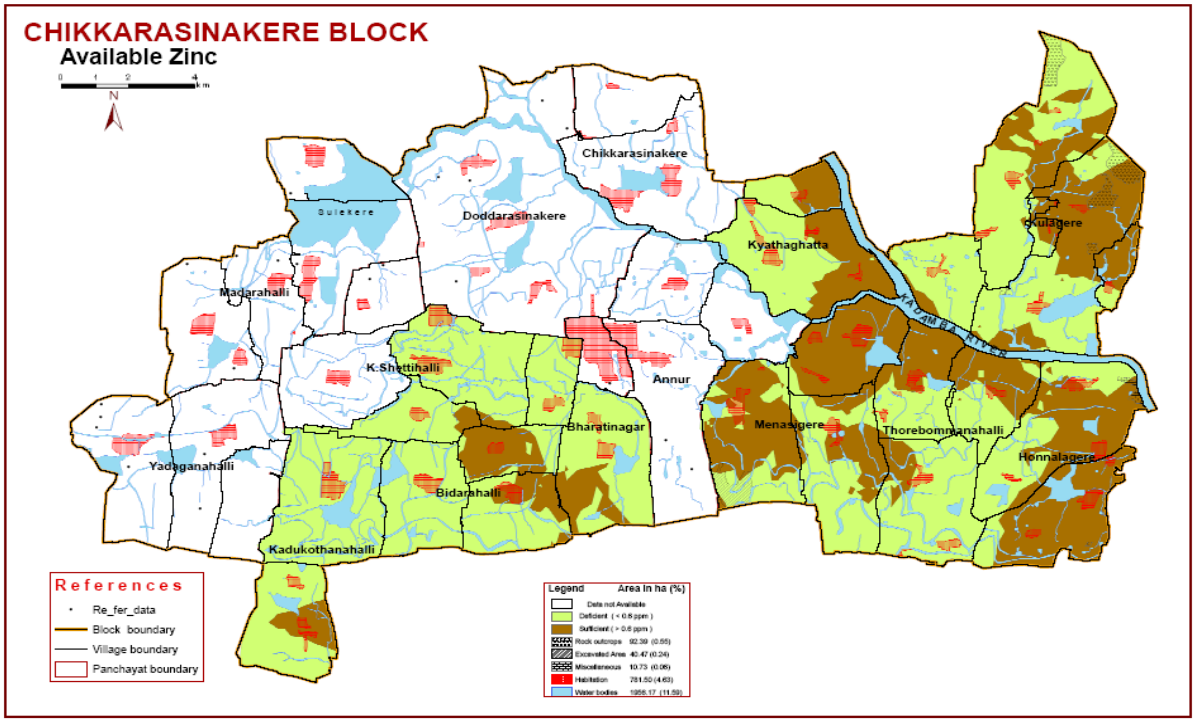

Fig. 2. Available Zinc status in Chikkarasinkere hobli

CONCLUSION

From the study it can be concluded that the availability of micronutrients in soils significantly influenced by soil properties viz. textural separates, organic carbon, $CaCO_3$, CEC and pH of soils. The DTPA-Zn consistently decreased with increasing depth of soil profile. The available iron significantly increased with increase in clay. Majority of the soils under study area were found deficient in available zinc. Available iron, copper and manganese were sufficient to adequate. One can also state that, judicious application of chemical fertilizers may help to maintain soil quality and productivity.

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RESEARCH ARTICLE

GENE ACTION STUDIES FOR SEED YIELD AND OTHER QUANTATIVE CHARACTERS IN FIELD PEA *(PISUM SATIVUM* L.)

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Abstract: In the present study, generation mean analysis were undertaken to estimate the nature and magnitude of gene action for yield and its component traits in two crosses of field pea viz IM 9214-10 X Rachna (C-1) and IM 9214-10 X Ambika (C-2). Scaling tests revealed the presence of one or more kinds of epistatic effects for almost all the agro-morphological traits. The selection of elite lines from delayed generations and subsequent inter mating might be useful approach to recover/ develop the high yielding field pea lines. The elite lines recovered from crosses IM 9214-10 X Rachna might be superior in terms of early maturity with more number of clusters per plant and seed yield per plant. Likewise, crosses *i.e.* IM 9214-10 X Ambika for plant height, number of clusters per plant and seed yield per plant; may give opportunity to isolate transgressive segregants in advanced generations.

Keywords: Epistasis, GMA, Gene effect, Inheritance, Field pea, Transgressive segregants

INTRODUCTION

Field pea is an important rabi season legume. Among the major pulse crops grown in India, field pea or dry pea *(Pisum sativum* L.) belongs to family leguminoceae and sub family Papilionaceae is considered to be the native of Ethiopia, the Mediterranean and Central Asia. It is a nutritious and protein rich (19.6%) crop, mostly used for green and dry seeds. Hence, pea is categorized as vegetable type and field pea. The area of field pea in India is about 0.76 million hectares with annual production of 0.84 million tones and productivity of 1100 kg/ha. During the past two decades, a number of varieties with high yield potential increased field pea productivity and it is highest among pulse crops grown in india. But if we compare the productivity of this crop with that in other countries, there is enough scope to future enhance its production and productivity in India Dixit *et al.* (2006).

The farmers of the state are small and marginal hence, there is urgent need to give them varieties which yield better even under average agronomic management. Dwarf type has greater potential under one or two irrigations. Hence, there is need to combine together desirable gene(s) from tall and dwarf types for evolving high yielding, disease resistant and widely adopted varieties for the state of Tripura. To attain the goal, the information on genetic architecture of yield and its attributing traits is essentially needed. Hence, the present study has been undertaken to generate basic information in relation to genetic improvement in seed yield.

The precise knowledge of the nature of gene action for yielding attributing traits help in the choice of an effective breeding strategy to accelerate the pace of genetics improvement of grain yield. Due to complex inheritance of seed yield and its component traits, development of high yielding field pea varieties may be possible by studying the nature and magnitude of genetic variability present in the available stocks for different traits. The adequate information on extent of variability parameters may be helpful in the development of promising varieties through identification of yield determinants Singh *et al.* (2016). The choice of efficient breeding programmes depends on knowledge of gene action involved in expression of yield and its component traits. Several researchers Ullah *et al.* (2011); Singh *et al.* (2014a, b) studied the genetic parameters and found additive type of gene action in governing the seed yield per plant (SYP), whereas Mehandi *et al.* (2013); Bisht *et al.* (2014) observed both additive and non-additive type of gene action. Patil *et al.* (2011) performed the combining ability analysis and suggested the importance of both additive and non-additive type of gene action for SYP and its other related traits. But these methods give general idea about inheritance of traits and some time misleads. Therefore, generation mean analysis was used in present study, which may give more reliable results about inheritance of traits due to individual cross analysis. Knowledge of genetic variability and genetic nature of characters under improvement is essential and pre-requisite for launching any breeding programme to achieve the goal. Genetic improvement in relation to grain yield and harvest index is prime objective in this crop. However, yield is a complex character contributed by several morpho-physiological traits (Singh *et al*,.2016) Hence, the knowledge relating genetic control of yield and its contributing traits is of immense use for initiating an efficient selection scheme for selecting a superior desirable genotype used in field pea breeding program for improving the seed physical quality. Keeping the above facts in mind, the present experiment was conducted (1) to

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test suitability of additive-dominance model and (2) to estimate genetic parameters such as gene effects using six basic generations in field pea.

MATERIAL AND METHOD

Genetics of seed yield and other traits of field pea were studied using the F_1, F_2, Bc_1P_1 (BC_1) and Bc_1P_2 (BC_2) of a cross between IM 9214-10 (Dwarf) as female parent (P_1) and Rachna (Tall) and Ambika (Tall) as male parents (P_2). The experiment was laid out in randomized block design (RBD) with three replications during rabi, 2013. These parents were selected from previous experiment conducted during rabi, 2012 (Singh *et al.*, 2014a) and crossed to obtain the crosses during, 2011. The F_1 seeds were subjected to back crossing and selfing during rabi, 2012. Ten competitive random plants from P_1, P_2 and F_1; 15 from BC_1 and BC_2 and 60 from F_2 population were randomly selected from each family in each replication, to record the observations for agro-morphological traits *viz.*, for days to first flowering, number of branches per plant, days to maturity, plant height, number of clusters per plant, pod bearing length, seed setting percent, pods per cluster, number of pods per plant, pod length, hundred seed weight and seed yield per plant. The traits *viz.*, days to first flower open and days to maturity were computed on plot basis. The observed means of the six generations and their standard errors for all the 12 characters in two crosses to test the adequacy of the additive dominance model were used to estimate the mid-parent [m], additive [d] and dominance [h] gene effects using the scaling test of Cavalli (1952). The gene effects and interactions for each characters were estimated after Hayman (1958). The significance of genetic parameters (m, [d], [h], [i], [j] and [l]) were tested using *t*-test. The data were subjected to generation mean analysis by using statistical package WINDOSTAT 9.1 version.

RESULT

The generation performance for crosses IM 9214-10 X Rachna and IM 9214-10 X Ambika are presented in Table 1. Components of mean *viz.*,constant mean (m), additive gene effects (d) and dominance gene effects (h) were estimated by using generation means. First three Parameter model was used and wherever it failed, six parameter model was applied for estimation of epistasis. Intra-allelic interactions *viz.*, additive x additive (i), additive × dominance (j) and dominance × dominance (l) were estimated and presented in Table 2. In cross IM 9214-10 X Rachna both additive and dominance gene effects irrespective of sign were significant for plant height, pod bearing length, seed setting percent, pods per cluster and pods per plant. However, clusters per plant showed significance of additive gene effects only. Whereas dominance was found to be significant for hundred seed weight and seed yield per plant. The additive effects were negative for plant height, pod bearing length, pods per cluster and pods per plant. On the other hand seed setting percent and pods per plant showed significance of negative dominance effects. Relatively dominance effect was greater for almost all the characters under study.

Inadequacy of additive-dominance model showed presence of epistasis for all the characters. All three types of interactions were significant for seven characters *viz.*, plant height, clusters per plant, pod bearing length, seed setting percent, pods per plant, hundred seed weight and seed yield per plant. Additive x dominance interaction was found to be positive and significant for days to maturity where as dominance × dominance gene interaction was found to be negative and significant for days to first flowering. All allelic and non allelic gene effects were non significant for branches per plant.

Likewise in cross IM 9214-10 X Ambika both additive and dominance gene effects were significant for plant height, clusters per plant, seed setting percent, pods per plant, hundred seed weight and seed yield per plant, whereas, dominance effect alone was significant for branches per plant days to maturity and pod bearing length. The additive effects in general were negative for all the characters except days to first flowering, days to maturity, pod bearing length and pods per plant. On the other hand dominance gene effects in general, were positive for almost all the traits except days to first flowering, days to maturity and pod bearing length. Relatively dominance effects were greater for almost all the characters under study.

Inadequacy of additive-dominance model showed presence of epistasis for all the characters. All the three types of interactions were recorded to be significant for eight characters viz., plant height, clusters per plant, pod bearing length, seed setting percent, pods per plant, hundred seed weight and seed yield per plant. Both additive x additive and additive x dominance interactions were found to be significant for days to maturity, whereas additive x additive along with dominance x dominance gene interactions were significant for branches per plant. All the allelic and non allelic gene effects were non significant for days to first flowering, pods per cluster and pod length.

DISCUSSION

The present study was planned to estimate the nature and magnitude of allelic and non allelic interactions in field pea. Three elite genotypes differing in many quantitative characters were crossed in two combinations to generate variability for different traits. Six generations of each of these crosses were grown and observations recorded on twelve important characters. The discussion on the results obtained with regards to

nature of gene action are discussed here cross and character wise.

In cross IM 9214-10 × Rachna both additive and dominance gene effect irrespective of the sign were significant for plant height, pod bearing length, seed setting percent, pods per cluster and pods per plant (Table 2). However, for clusters per plant only additive effects; for hundred seed weight and seed yield per plant only dominant gene effects were found to be important. Presence of epistasis was recorded for all twelve characters in both the crosses (Table 2). Among non allelic interactions, all three types of interactions were found significant for plant height, clusters per plant, pod bearing length, seed setting percent, pods per plant, hundred seed weight and seed yield per plant whereas, for days to maturity, additive × dominance and for days to first flowering, dominance × dominance type of interactions were found significant. Duplicate type of epistasis (dissimilar sign of h & l) was observed for days to first flowering, branches per plant, plant height, pod bearing length, seed setting percent, pods per plant and hundred seed weight while, complementary epistasis (similar sign of h and l) was observed for days to maturity, clusters per plant, pod length and seed yield per plant.

The additive gene effects and additive × additive or any digenic complementary gene interaction are fixable and useful. In these populations complementary epistasis could be exploited for the improvement of clusters per plant and seed yield per plant. Hence direct selection for these traits could be beneficial but with proper care otherwise predominance of dominant and dominant × dominant gene effects may mislead the selection.

In the cross IM 9214-10 × Ambika both additive and dominance gene effects were found significant for plant height, clusters per plant, seed setting percent, pods per plant, hundred seed weight and seed yield per plant. Dominance gene effects alone were significant for branches per plant, days to maturity and pod bearing length. Interaction effects were found significant for all the characters except days to first flowering and pod length. Both additive x additive and dominance x dominance type of interaction effects were significant for branches per plant. All three types of interaction effects were found significant for days to maturity, plant height, clusters per plant, pod bearing length, seed setting percent, pods per plant, hundred seed weight and seed yield per plant. except for plant height. Clusters per plant and seed yield per plant, duplicate epistasis was found for all characters. Presence of complementary type of epistasis was observed for plant height, clusters per plant and seed yield per plant which could be exploited for improvement of grain yield in the populations under study.

Table 1. Cross wise mean performance of different generations for yield and attributes in field pea

	DFF	NBP	DM	PH	NCP	PBL	SSP	PPC	NPP	PL	SI	SYP
C-1: IM 9214-10 × RACHNA												
P_1	43.73	3.67	121.80	60.00	4.67	7.33	68.67	1.20	8.00	4.33	18.73	5.17
P_2	42.20	3.27	125.20	76.00	4.80	18.07	72.97	1.40	9.93	4.73	19.43	5.90
F_1	42.40	3.67	123.53	79.67	8.80	19.33	75.33	1.53	19.03	4.33	17.70	12.07
F_1	44.00	3.23	124.33	63.44	6.73	17.80	73.77	1.37	17.00	4.83	18.65	6.47
BC_1	44.30	3.20	123.87	79.60	6.27	17.87	77.67	1.40	8.47	4.67	20.40	8.07
BC_2	44.33	3.13	123.80	85.07	5.33	20.53	69.33	1.47	10.60	4.73	19.85	6.33
C-2: IM 9214-10 × AMBIKA												
P_1	45.43	3.47	120.87	63.20	4.60	8.00	69.00	1.23	8.60	4.40	20.03	5.62
P_2	45.13	3.67	124.20	88.67	4.47	19.53	69.67	1.43	16.33	4.70	19.28	6.40
F_1	43.27	3.27	123.47	96.20	8.30	15.33	69.83	1.37	12.83	4.67	20.20	9.53
F_1	43.47	3.13	124.60	65.33	4.13	15.83	72.17	1.33	9.37	4.50	19.81	6.70
BC_1	43.80	3.53	124.03	64.17	4.50	14.00	72.33	1.43	13.70	4.43	20.73	8.30
BC_2	43.33	3.70	123.33	71.47	5.17	13.73	76.17	1.47	9.93	4.80	21.73	10.00

DFF=Days to first flowering, NBP=No. of branches/plant, DM=Days to maturity, PH=Plant height, NCP= No. of cluster/plant,PBL= Pod bearing length, SSP=Seed setting percent, PPC=Pods/cluster, NPP=No. of pods per plant, PL=Pod length, SI=Seed index, SYP=seed yield/plant

Table 2. Estimates of gene effects and their standard errors for different characters in field pea (*Pisum sativum* L.) Cross-1

Characters	m	d	h	i	j	l	Type of epistasis
First flower (days)	44.00±0.11**	0.06±0.52	0.76±1.18	1.33±1.14	-0.76±.55	-7.93±2.22**	D
Branches /plant	3.23±.14	0.067±0.17	-0.050±.73	-0.267±.679	-0.116±.210	1.83±1.05	D
Maturity (days)	124.33±.29**	0.067±369	1.96±1.41	1.99±1.37	1.76±.45**	0.73±1.99	C
Plant height (cm)	63.44±47**	-5.46±.92**	99.57±2.69**	75.57±2.64**	14.86±.99**	-134.2±4.2**	D
Clusters/plant	6.73±.266**	0.93±.22**	0.33±1.18	-3.73±1.15**	0.99±.27**	7.60±1.47**	C
Pod bearing length (cm)	17.80±2.00**	-2.66±.45**	12.23±1.27**	5.59±1.21**	2.70±.49**	-18.33±2.1**	C
Seed setting (%)	73.76±.95**	3.33±.94**	-6.25±5.29**	-11.0±4.27**	5.78±.94**	18.76±5.42**	D
Pods /cluster	1.36±.033**	-0.06±.033**	0.48±.178**	.244±.149	0.049±.055	0.300±.272	D
Pods /plant	17.00±.20**	-2.13±.35**	-19.79±1.1**	-29.86±1.0**	-1.16±.390**	47.73±1.73**	D
Pod length(cm)	4.80±.166**	-0.066±.159	-0.73±.74	-0.53±.739	0.133±.166	-0.53±.94	D
hundred seed weight (g)	18.64±.086**	0.55±.368	4.52±.85**	5.90±.813**	0.90±.40**	-12.83±1.6**	C
Seed yield/plant	6.46±.088**	1.73±.124	9.46±679**	2.93±.43**	2.10±.138**	3.46±1.23**	D

Cross-2

Characters	m	d	h	i	j	l	Type of epistasis
First flower (days)	43.46±.067**	0.46±.58	-1.61±1.35	0.40±1.19	0.316±.62	2.43±2.66	D
Branches /plant	3.13±.133**	-0.16±.176	1.63±.74**	1.93±.64**	0.066±.22	-2.73±1.16*	D
Maturity (days)	124.60±.30**	0.699±.691	-2.73±1.98**	-3.66±1.84**	2.36±.834**	0.93±3.35	C
Plant height (cm)	65.33±.72**	-7.3±.52**	44.69±3.14**	9.93±3.08**	19.93±.59**	34.06±3.78**	D
Clusters/plant	4.13±.13**	-.66±.105**	6.56±.605**	2.80±.573**	-0.73±.159**	3.53±.783**	C
Pod bearing length (cm)	15.83±.84**	0.26±.63	-6.09±3.61**	-7.86±3.59**	6.03±.674**	11.00±4.28**	C
Seed setting (%)	72.16±1.08**	-3.83±.745**	8.83±4.70**	8.33±4.58**	-3.49±.816**	-27.0±5.61**	D
Pods /cluster	1.33±.033**	-0.033±.137	0.500±.308	0.46±.305	0.066±.139	-0.86±.57	D
Pods /plant	9.36±.712**	3.76±.517**	10.16±3.10**	9.80±3.03**	7.63±.64**	-6.46±1.99**	D
Pod length(cm)	4.50±.28**	-0.36±.21	0.58±1.24	0.46±1.2	-0.21±.23	-0.49±1.48	D
hundred seed weight (g)	19.81±.013**	-0.99±.363**	6.22±.84**	5.67±.72**	-1.37±.39**	-10.9±1.68**	C
Seed yield/plant	6.70±.25**	-1.70±.25**	13.32±1.16**	9.7±1.13**	-1.31±.28**	15.31±1.53**	D

*, ** Significant at 5 and 1 percent level of significance, C= Similar sign of h & l, D= Dissimilar sign of h & l

The [h] gene effects were greater than the [d] gene effects for all agro-morphological traits in both crosses, indicated the importance of dominance gene effects for yield and its related agro-morphological traits. The contribution of dominance gene effects varied with to cross and traits. Similar result was also observed earlier by Gawande *et al.* (2005) and Azizi *et al.* (2006). The negative and positive sign of [h] gene effects is a function of the F_1 mean value in relation to mid parent heterosis contributing to dominance gene effects (Cukadar-Olmedo and Miller, 1997). It is possible that the epistasis significantly contributed to genetic variance. Beside the additive and dominance genetic effects, epistasis components have also contributed to genetic variation with different magnitude for most of the yield and yield component traits. In such situation, the appropriate breeding method can effectively exploit the three types of gene effects.

The results obtained from present investigation reveal that seed yield in these populations were under the control of both additive and dominance gene effects. However, in both the crosses, dominance genetic variance was more prominent for seed yield. Hence careful selection for superior single plants should be operated carefully in segregating generations. Simultaneously inter-mating among the superior segregants can also be practiced for accumulating desirable genes for higher seed yield and other traits.

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RESEARCH ARTICLE

NUTRIENT UPTAKE BY WEEDS AND PEA (*PISUM SATIVUM* L.) AS INFLENCED BY DIFFERENT HERBICIDE COMBINATIONS

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Abstract: A field experiment was carried out during the winter season of 2012-13 and 2013-14 at Palampur to evolve an effective herbicide combination on nutrient depletion by weeds in pea (*Pisum sativum* L.). In the present study, pendimethalin 1000 g/ha *fb* HW (45 DAS)andpendimethalin 1000 g/ha (Pre)*fb* imazethapyr + imazamox 60 g/ha (45 DAS) resulted in significantly lower total weed dry weight over other herbicidal treatments.All the herbicide combinations were comparable to weed free in reducing the GR_w between 90-120 DAS. Pendimethalin 1000 g/ha *fb* HW (45 DAS), pendimethalin 1000 g/ha *fb* imazethapyr + imazamox 60 g/ha (45 DAS) were as effective as weed free in reducing NPK uptake by weeds. Weeds in weedy check removed 49.3 kg/ha N, 19.7 kg/ha P and 44.7 kg/ha K depriving thereby the crop for that much amount of nutrients. Most of the treatments were results in significantly higher crop dry matter accumulation. Significantly higher green pod yield and NPK uptake by crop were obtained in weed free, pendimethalin 1000 g/ha *fb* HW **(**45 DAS) and pendimethalin 1000 g/ha *fb* imazethapyr + imazamox 60 g/ha (45 DAS) treatments.Herbicide combinations in general were better than sole application of herbicides in effectively reducing the NPK uptake by weeds and increasing NPK uptake by crop.

Keywords: Hand weeding, Imazethapyr, Nutrient uptake, Peas, Pendimethalin

INTRODUCTION

A strong competition is going on between weeds and crop plants for nutrients, and that is the most critical factor in the first period of the vegetation. Plants compete mainly for the sufficient amount of macronutrients, for nitrogen, phosphorus, and potassium. Most weed species can take up nitrogen and potassium from soil at a higher degree than crop plant living in association with it. Pea is one crop, which builds up the soil fertility by atmospheric nitrogen fixation through the root nodules. Pea has great potential as an exceptionally nutritive and very rich protein food. However, it has higher requirement of phosphorus for symbiotic nitrogen fixation. Among different production factors, weeds pose serious threat to the productivity of garden pea. However, weeds are the major threat in harnessing the full potential of applied and native plant nutrients. They remove considerable amount of nutrients and adversely affect the yield of the crop (Kumar *et al*., 2005; Dubey *et al*.1999). Weeds have been reported to cause 56.8-81(%) losses in its yield (Rana *et al.,*2013; Singh *et al.,*1996) under different agro-climatic conditions. ln Himachal Pradesh, pea crop has been reported to be infested with a variety of weeds *viz*., *Phalaris minor, Avena ludoviciana, Loliumtemulentum, Vicia sativa and Anagallis arvensis* (Rana *et al.,* 2013). In order to achieve enhanced crop production and higher benefits from applied inputs, there must be a strong weed management strategy.They can be controlled by manual, mechanical and chemical methods. Manual method of weed control is labour intensive, cumbersome and time consuming. Whereas, mechanical methods of weed control are reported to cause injury to root system (Casarini *et al.,* 1996). Various pre-plant incorporation and pre-emergence herbicides have been tested and recommended under different agro-climatic conditions of Himachal Pradesh (Singh *et al.,*1996). However, the information on post-emergence herbicides to control weeds is scanty. Many a times extension workers and farmers of the state demand information on post emergence herbicides particularly when they fail to advocate/apply pre-emergence herbicides due to one or the other reason. Post-emergence herbicides are also required when pre-emergence fail to give satisfactory weed control. New post-emergence herbicides *viz.*, imazethapyr alone and in combination with imazamox (odissey) have been introduced. Therefore, the present investigation was carried out for having an effective management strategy for season long control of weeds in pea under mid hill conditions of Himachal Pradesh.

MATERIAL AND METHOD

Pea variety ‘Palam Priya’ was sown during the second fortnight of October for two consecutive years (2012-12 and 2013-14) with recommended package of practices except weed control. Twelve weed control treatments *viz.,* pendimethalin 1500 g/ha pre emergence, pendimethalin 1000 g/ha (Pre)*fb* imazethapyr 100 g/ha (45 DAS), imazethapyr 100 g/ha (Pre)*fb* imazethapyr 100 g/ha (45 DAS), imazethapyr + pendimethalin 1200 g/ha pre emergence, imazethapyr + pendimethalin 1500 g/ha pre emergence, imazethapyr + pendimethalin 1000 g/ha (Pre)*fb* imazethapyr 100 g/ha (45 DAS),

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imazethapyr + imazamox 60 g/ha (45 DAS), imazethapyr + imazamox 90 g/ha (45 DAS), pendimethalin 1000 g/ha (Pre)*fb* imazethapyr + imazamox 60 g/ha (45 DAS), pendimethalin1000 g/ha (Pre)*fb* 1HW (45 DAS), weed free and weedy check were tested in a Randomized Block Design with three replications. Soil of the test site was silty clay loam in texture, acidic in reaction, medium in available nitrogen(322.9 kg/ha) and K (276.4 kg/ha) and high in available P (25.8 kg/ha).Observation on weed density and biomass were recorded at 60, 90, 120 DAS and at harvest using quadrat of 0.5 m x 0.5 m, placed at two random spot. The crop was harvested on April 20 during the first year and April 24 during the second year. Yields were harvested from net plot in four picking. Weed biomass data showed variation and were subjected to square root transformation [$(\sqrt{x+1})$].Weed control index was worked out based on weed dry weight.

$$\text{Weed Control index (\%)} = \frac{\text{Wc} - \text{Wt}}{\text{Wc}} \text{X } 100$$

Where,

Wc- Weed dry weight (g/m^2) in control plot and

Wt- Weed dry weight (g/m^2) in treated plot.

$$\text{Weed index (\%)} = \frac{\text{X} - \text{Y}}{\text{X}} \text{X } 100$$

Where,

X - Yield from weed free treatment

Y - Yield of particular treatment for which WI is to be worked out.

$$\text{GRw } (g/m^2/\text{day}) = \frac{W_2 - W_1}{t_2 - t_1}$$

$$\text{RGRw } (g/g/\text{day}) = \frac{\text{Log}_e W_2 - \log_e W_1}{t_2 - t_1}$$

Where, W_2 and W_1 are the total dry weight at times t_2 and t_1, respectively.

RESULT AND DISCUSSION

Effect on weeds

The major weed flora of the experimental field was composed of *Phalaris minor* (28.8%), *Alopecurus myosuroides* (21.3%),*Avena ludoviciana* (15.8%), *Lolium temulentum* (12.1%) and *Vicia sativa* (16.7%).Among other weeds, *Stellaria media*, *Poa annua, Anagallis arvensis* and *Coronopus didymus* showed their little infestation.

Data pertaining to progressive dry matter accumulation by weeds have been presented in Table 1. The data revealed that in general, dry matter accumulation increased consistently up to 120 DAS, thereafter it declined gradually. The decline in weed dry weight was owed to withering of weeds. Data on weed dry weight at maximum dry matter stage *i.e.* 120 DAS have been given in Table 1. Weed control treatments significantly decreased total weed dry weight as compared to weedy check. Removing the weeds whenever they appear under the weed free treatment resulted in complete elimination of weed competition as it resulted in lowest total weed dry weight. Pendimethalin 1000 g/ha *fb* HW (45 DAS)being at par withpendimethalin 1000 g/ha (Pre)*fb* imazethapyr + imazamox 60 g/ha (45 DAS) resulted in significantly lower total weed dry weight over other herbicidal treatments. The superiority of pendimethalin *fb* HW in controlling weeds has been reported by Kumar and Singh (1994). Imazethapyr + imazamox 60 g/ha (45 DAS), imazethapyr + pendimethalin 1000 g/ha *fb* imazethapyr 100 g/ha (45 DAS), imazethapyr + pendimethalin 1200 g/ha pre emergence**,** imazethapyr + imazamox 90 g/ha (45 DAS) and pendimethalin 1000 g/ha *fb* imazethapyr 100 g/ha (45 DAS) behaving statistically alike were the next better treatments. Owing to synergetic, enhancement or additive effects, herbicidal combinations in general were better than sole application of herbicides in effectively reducing the total weed dry weight.

Species-wise better control of weeds under the herbicide mixture or sequence application, weed control index under them was comparable to weed free. Application of herbicide alone gave poor control of weeds, therefore had lower weed control index.

The data on effect of treatments on nutrient uptake by weeds has been embodied in Table 3. Owing to significant reduction in dry weight, all weed control treatments significantly reduced N, P and K uptake by weeds as compared to weedy check. Pendimethalin 1000 g/ha *fb* HW (45 DAS), pendimethalin 1000 g/ha *fb* imazethapyr + imazamox 60 g/ha (45 DAS) were as effective as weed free in reducing N, P and K uptake by weeds. In general, all herbicide combinations proved superior to alone application of herbicides in reducing the N, P and K uptake by weeds. Weeds in weedy check removed 49.3 kg/ha N, 19.7 kg/ha P and 44.7 kg/ha K depriving thereby the crop for that much amount of nutrients. Similar results have been reported by Wagner and Nadasy (2007).

Effect on crop

The trend in progressive dry matter accumulation by pea crop under different weed control treatments has been shown graphically in Fig. 1. Dry matter accumulation increased consistently with the advancement of crop growth. The data on total dry matter accumulation by pea crop at final harvest as influenced by different treatments have been given in Table 2. A cursory glance at the data depicts that the plant dry weight increased consistently with advancement in crop growth with maximum rate at 90 to 120 DAS. Pendimethalin 1000 g/ha *fb* HW (45 DAS) and weed free remaining statistically at par with pendimethalin 1000 g/ha (Pre)*fb* imazethapyr + imazamox 60 g/ha (45 DAS), imazethapyr + pendimethalin 1000 g/ha *fb* imazethapyr 100 g/ha (45 DAS), imazethapyr + imazamox 90 g/ha (45 DAS),

imazethapyr + imazamox 60 g/ha (45 DAS), imazethapyr + pendimethalin 1200 g/ha pre emergence and pendimethalin 1000 g/ha *fb* imazethapyr 100 g/ha (45 DAS) resulted in significantly higher dry matter accumulation over rest of the treatments. The reduction in population and dry weight of weeds under these treatments and higher weed control index created favourable micro-environment for growth and development of pea crop and thus increased the dry matter accumulation of pea.

Data pertaining to crop growth rate (CGR) and relative growth rate (RGR) of pea crop have been embodied in Table 2. Weed control treatments did not significantly influence the CGR and RGR of pea. This showed that rate of growth of pea remained unaffected irrespective to variation in population and dry weight of weeds. However, data on number of days taken for attainment of various development stages *viz*., emergence count, 50% flowering, first picking and maturity was not significant in both the years of experimentation (Data not shown).

Table 1. Effect of weed control treatments on total weed dry weight (g/m^2) at different crop stage and weed control index (%)

Treatment	Dose (g/ha)	Time of application	Weed dry weight (DAS)				Weed control index (DAS)			
			60	90	120	At harvest	60	90	120	At harvest
Pendimethalin	1500	Pre emergence	8.3 (68.3)	11.0 (120.5)	12.1 (145.6)	10.1 (100.3)	58.2	55.4	50.2	49.2
Pendimethalin *fb* imazethapyr	1000 *fb* 100	Pre *fb* post (45 DAS)	7.6 (57.6)	9.4 (87.5)	10.7 (114.1)	8.6 (73.1)	64.7	67.7	61.0	63.0
Imazethapyr *fb* imazethapyr	100 *fb* 100	Pre *fb* post (45 DAS)	7.6 (56.5)	11.4 (129.1)	12.3 (149.3)	11.2 (124.8)	65.4	52.3	48.9	36.7
Imazethapyr + pendimethalin	1200	Pre emergence	6.2 (37.3)	9.4 (87.5)	10.2 (104.0)	10.2 (104.0)	77.1	67.7	64.4	47.3
Imazethapyr + pendimethalin	1500	Pre emergence	6.2 (37.9)	9.3 (84.8)	11.2 (124.8)	10.2 (102.4)	76.8	68.6	57.3	48.1
Imazethapyr + pendimethalin *fb* imazethapyr	1000 *fb* 100	Pre *fb* post (45 DAS)	4.2 (17.1)	8.3 (67.7)	10.2 (102.4)	7.1 (50.1)	89.5	75.0	65.0	74.6
Imazethapyr + imazamox	60	Post (45 DAS)	4.8 (22.4)	8.5 (70.9)	9.8 (96.0)	8.3 (68.8)	86.3	73.8	67.2	65.1
Imazethapyr + imazamox	90	Post (45 DAS)	6.3 (40.0)	8.9 (78.9)	10.5 (109.3)	9.6 (91.7)	75.5	70.8	62.6	53.5
Pendimethalin *fb* imazethapyr + imazamox	1000 *fb* 60	Pre *fb* post (45 DAS)	2.1 (4.3)	7.9 (61.3)	8.8 (76.3)	7.0 (47.5)	97.4	77.3	73.9	75.9
Pendimethalin *fb* 1HW	1000	Pre *fb* HW (45 DAS)	1.0 (0.0)	6.6 (43.2)	8.1 (65.1)	6.2 (37.9)	100.0	84.0	77.7	80.8
Weed free	-	-	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	1.0 (0.0)	100.0	100.0	100.0	100.0
Weedy check	-	-	12.8 (163.2)	16.5 (270.4)	17.1 (292.8)	14.1 (197.3)	0.0	0.0	0.0	0.0
SE(m+-)			0.48	0 .33	0.41	0.37	-	-	-	-
CD (P=0.05)			1.0	0.7	0.9	0.8	-	-	-	-

Table 2. Effect of weed control treatments on total crop dry matter accumulation (g/m^2), crop growth analysis, yield and weed index

Treatment	Dose (g/ha)	Time of application	Crop dry matter (At harvest)	CGR (g/m^2/day) 90-120 DAS	RGR_c (mg/g/day) 90-120 DAS	Grain yield (t/ha)			WI (%)	Straw yield (t/ha)		
						2012-13	2103-14	Mean		2012-13	2103-14	Mean
Pendimethalin	1500	Pre emergence	343.7	4.049	21.45	6.57	6.57	6.6	9.9	2.11	1.75	1.9
Pendimethalin *fb* imazethapyr	1000 *fb* 100	Pre *fb* post (45 DAS)	351.1	3.975	20.73	6.29	6.49	6.4	12.3	2.07	1.87	2.0
Imazethapyr *fb* imazethapyr	100 *fb* 100	Pre *fb* post (45 DAS)	346.7	3.852	20.02	6.21	6.37	6.3	13.7	2.03	1.71	1.9
Imazethapyr + pendimethalin	1200	Pre emergence	353.3	4.025	21.31	5.97	6.25	6.1	16.2	2.11	1.83	2.0
Imazethapyr + pendimethalin	1500	Pre emergence	344.4	4.074	21.55	6.13	6.41	6.3	14.0	2.03	1.79	1.9
Imazethapyr + pendimethalin *fb* imazethapyr	1000 *fb* 100	Pre *fb* post (45 DAS)	389.6	3.901	20.04	6.09	6.81	6.5	11.5	1.99	1.99	2.0
Imazethapyr + imazamox	60	Post (45 DAS)	384.4	3.778	19.31	6.01	6.69	6.4	12.9	2.11	1.91	2.0

Imazethapyr + imazamox	90	Post (45 DAS)	385.2	3.827	19.68	6.53	6.81	6.7	8.5	2.15	1.95	2.1
Pendimethalin *fb* imazethapyr + imazamox	1000 *fb* 60	Pre *fb* Post (45 DAS)	396.3	4.114	21.54	7.01	7.25	7.1	2.2	2.23	2.07	2.2
Pendimethalin *fb* 1HW	1000	Pre *fb* HW (45 DAS)	400.0	4.136	21.32	7.17	7.33	7.2	0.5	2.27	2.11	2.2
Weed free	-	-	403.7	4.296	21.16	7.21	7.37	7.3	0.0	2.35	2.11	2.2
Weedy check	-	-	188.9	1.012	3.20	4.34	4.74	4.5	37.7	1.87	1.71	1.8
SE(m+-)			27.24	0.80	5.58	0.26	0.43	0.32	-	0.10	0.11	0.07
CD (P=0.05)			56.8	1.686	NS	0.56	0.90	0.7	-	0.21	0.22	0.2

Table 3. Effect of weed control treatments on nutrient uptake by weeds and crop (kg/ha)

Treatment	Dose (g/ha)	Time of application	Weeds			Crop								
			N	P	K	N			P			K		
						Pods	Straw	Total	Pods	Straw	Total	Pods	Straw	Total
Pendimethalin	1500	Pre emergence	20.1	7.0	21.7	57.2	42.6	99.8	7.9	7.6	15.5	41.4	42.6	84.0
Pendimethalin *fb* imazethapyr	1000 *fb* 100	Pre *fb* post (45 DAS)	14.1	4.6	14.6	58.4	43.1	101.5	7.8	7.5	15.3	42.8	48.0	90.9
Imazethapyr *fb* imazethapyr	100 *fb* 100	Pre *fb* post (45 DAS)	25.8	9.6	27.5	56.7	42.2	99.0	7.6	6.8	14.5	38.2	43.4	81.6
Imazethapyr + pendimethalin	1200	Pre emergence	19.8	6.2	18.7	54.4	45.2	99.6	9.4	7.9	17.3	40.0	45.2	85.2
Imazethapyr + pendi methalin	1500	Pre emergence	17.4	5.8	18.4	57.1	44.2	101.3	8.3	6.6	14.9	40.4	45.4	85.8
Imazethapyr + pendimethalin *fb* imazethapyr	1000 *fb* 100	Pre *fb* post (45 DAS)	8.2	2.5	8.7	62.0	47.8	109.8	10.2	8.6	18.8	42.9	51.8	94.7
Imazethapyr + imazamox	60	Post (45 DAS)	11.0	3.7	12.2	61.6	46.5	108.1	8.7	7.0	15.7	42.8	49.7	92.5
Imazethapyr + imazamox	90	Post (45 DAS)	15.3	4.6	15.3	62.7	46.8	109.5	10.2	8.5	18.7	44.3	50.1	94.4
Pendimethalin *fb* imazethapyr + imazamox	1000 *fb* 60	Pre *fb* post (45 DAS)	7.4	2.2	7.3	67.4	49.7	117.1	13.0	10.4	23.4	54.4	54.5	108.9
Pendimethalin *fb* 1HW	1000	Pre *fb* HW (45 DAS)	5.8	1.4	5.7	68.9	51.4	120.2	13.2	10.6	23.7	54.2	56.3	110.5
Weed free	-	-	0.0	0.0	0.0	70.0	52.1	122.1	14.0	10.6	24.6	55.3	57.0	112.2
Weedy check	-	-	49.3	19.7	44.7	45.5	43.4	88.9	5.7	7.4	13.1	26.5	43.5	70.1
SE(m+-)			2.32	0.51	2.17	2.4	2.13	3.18	0.97	1.20	1.88	3.35	2.96	4.39
CD (P=0.05)			4.9	1.1	4.5	5.1	4.5	6.6	2.0	2.5	3.9	7.0	6.2	9.2

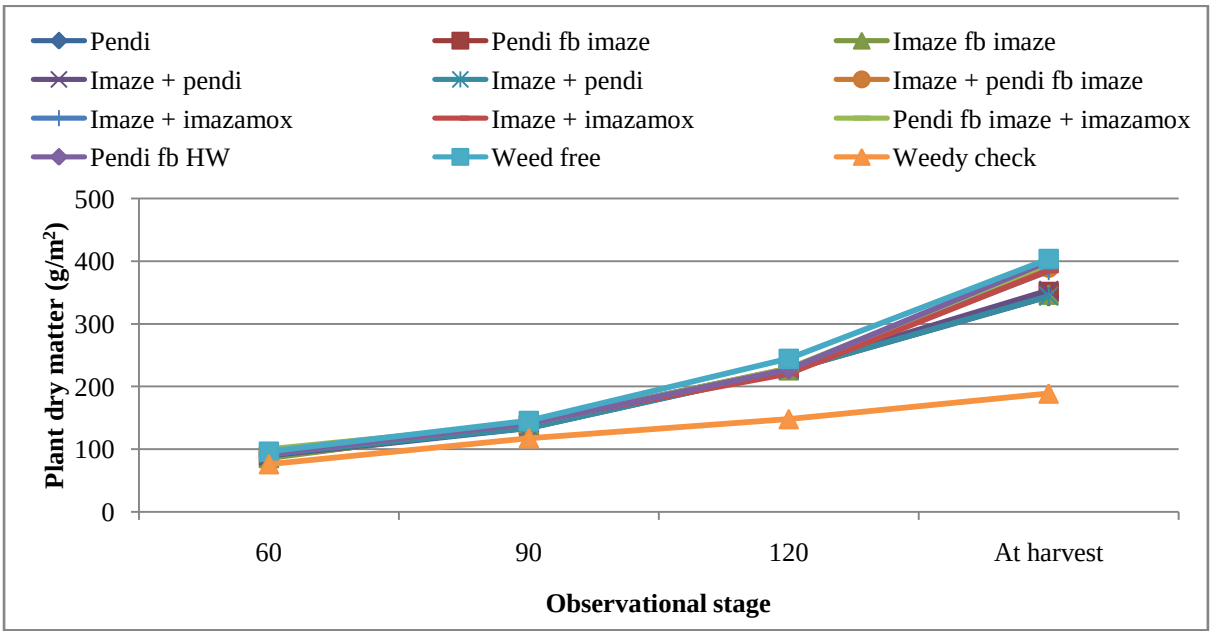

Fig 1. Effect of weed control treatments on progressive dry matter accumulation by pea at different stages of observation

Weed control treatments brought about significant variation in green pod yield (Table 2). All weed control treatments were significantly superior to weedy check in influencing green pod yield. Significantly higher green pod yield was obtained in weed free, pendimethalin 1000 g/ha *fb* HW **(**45 DAS) and pendimethalin 1000 g/ha *fb* imazethapyr + imazamox 60 g/ha (45 DAS) treatments. Imazethapyr + imazamox 90 g/ha (45 DAS) and imazethapyr + pendimethalin 1000 g/ha *fb* imazethapyr 100 g/ha (45 DAS) being statically similar with each other were the next superior treatments in influencing green pod yield. Similarly observation with respect to pendimethalin *fb* HW on yield attributes and yield were recorded (Vaishya *et al.*,1999; Tripathi *et al.*,1993; Kumar and Singh 1994). As indicated by weed index, un-interrupted growth of weeds in the weedy check reduced pea yield by 37.7% over weed free.Significantly higher straw yield was obtained with weed free and pendimethalin 1000 g/ha *fb* HW (45 DAS). However, they behaved statically alike to pendimethalin 1000 g/ha *fb* imazethapyr + imazamox 60 g/ha (45 DAS), imazethapyr + pendimethalin 1000 g/ha *fb* imazethapyr 100 g/ha (45 DAS), imazethapyr + imazamox 90 g/ha (45 DAS) and imazethapyr + imazamox 60 g/ha (45 DAS). Unchecked weed growth reduced the straw yield to the extent of 18.2% as compared to best treatment *i.e*. pendimethalin 1000 g/ha *fb* imazethapyr + imazamox 60 g/ha (45 DAS).

The data on effect of treatments on N, P and K uptake by pea have been embodied in Table 3. All the weed control treatments significantly increased the N, P and K uptake by pea over weedy check. Because of the higher pea pod and straw yield, weed free remaining at par with pendimethalin 1000 g/ha *fb* HW (45 DAS), pendimethalin 1000 g/ha *fb* imazethapyr + imazamox 60 g/ha (45 DAS), imazethapyr + pendimethalin 1000 g/ha *fb* imazethapyr 100 g/ha (45 DAS), imazethapyr + imazamox 90 g/ha (45 DAS), imazethapyr + imazamox 60 g/ha (45 DAS), pendimethalin 1000 g/ha *fb* imazethapyr 100 g/ha (45 DAS), imazethapyr + pendimethalin 1500 g/ha pre emergence and imazethapyr + pendimethalin 1200 g/ha pre emergence resulted in significantly higher N uptake by crop. Imazethapyr 100 g/ha (Pre)*fb* imazethapyr 100 g/ha (45 DAS), imazethapyr + pendimethalin 1200 g/ha pre emergence and pendimethalin 1500 g/ha pre emergence were less effective treatment in influencing N uptake than other treatments.

Weed free remaining at par with pendimethalin 1000 g/ha *fb* HW (45 DAS), pendimethalin 1000 g/ha *fb* imazethapyr + imazamox 60 g/ha (45 DAS), imazethapyr + pendimethalin 1000 g/ha *fb* imazethapyr 100 g/ha (45 DAS) and imazethapyr + imazamox 90 g/ha (45 DAS) resulted in significantly higher P and K uptake by crop. In general, all herbicide combinations were superior to alone application of herbicides in improving the N, P and K uptake by crop. The superiority of herbicide combination in influencing N, P and K uptake by pea crop has been documented Ramia *et al.*,(2013). Weed free resulted in 37.3, 87.8 and 60.0 per cent higher N, P and K uptake over weedy check, respectively.

Application of pendimethalin 1000 g/ha (Pre)*fb* imazethapyr + imazamox 60 g/ha (45 DAS) reducing nutrient uptake by weeds, increased by pea and behaved statistically alike with weed free and pendimethalin 1000 g/ha *fb* HW (45 DAS). Thus, combination of herbicides (tank mixed or sequential) is the better option for the control of mixed weed flora to obtain higher yield in pea crop.

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RESEARCH ARTICLE

EFFECT OF THE NUTRIENT ON YIELD AND YIELD ATTRIBUTING CHARACTERS IN MAIZE CROP

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Abstract: The field experiment on maize crop was conducted at research farm of C.S.A. University Of Agriculture & Technology Kanpur, during Kharif season 2015-16 and 2016-17. The doses of experiment were Control, 100% NPK , 100% NPK+ S40, 100% NPK + Zn5, 100% NPK+S40 +Zn5, 125% NPK, 125% NPK + S40, 125% NPK + Zn5, 125% NPK + S40 + Zn5 and 150% NPK. The results showed that the grain yield of maize first year (2015) varied from 14.33 to 30.78 q ha^{-1} and in second year (2016) varied from 14.85 to 32.80 q ha^{-1} and the straw yield of maize first year (2015) varied from 38.91 to 81.10 q ha^{-1} and in second year (2016) varied from 39.48 to 82.13 q ha^{-1} . It was noted the number of plant in maize varied from 109.75 to 112.25 $plot^{-1}$ and 109.60 to 112.00 $plot^{-1}$, Plant height (cm) from 175.25 to 213.75 $plant^{-1}$ and 178.75 to 219.48 $plant^{-1}$, Number of cob from 1 to 1.48 and 1.10 to 1.45 $plant^{-1}$, length of cob from 13.40 to 22.45 and 14.60 to 23.49 cm $plant^{-1}$, Test weight from 20.28 g to 32.37 g 100 $grain^{-1}$ and 20.87 g to 33.01 g100 $grain^{-1}$ in first and second years, respectively.

Keywords: Maize, Nutrient, Crop, Production

INTRODUCTION

Maize (*Zea mays L.)* is becoming very popular cereal crop in India because of increasing market price and high production potential of hybrid varieties in both irrigated as well as rainfed conditions. Hence the trend of replacing some cash crops with maize in intensive cultivation is observed in present condition. Maize crop has better yield response to chemical or inorganic fertilizers. Hence heavy doses of their fertilizers are applied to maize. Though these practices help to increase the temporary increase the production of crop deterioration of natural resources (viz. land, water and air) is also the another side of such high input intensive cultivation. Efficiency of balanced use of fertilizers is an alternative for obtaining high sustainable yields.

Globally, maize is known as queen of cereals because it has the highest genetic yield potential among the cereals. It is cultivated on nearly 150 mha in about 160 countries having wider diversity of soil, climate, biodiversity and management practices that contributes 36% (782 mt) in the global grain production. The United state of America is the largest producer of maize contributes nearly 35% of the total production in the world and maize is the driving factor of the U.S. economy.

The cereals occupy about 54% of total cropped area in India. The India produces Maize occupies about 3.6% of the total cropped *area* of India. Maize is the third most important cereal crop in India after rice and wheat. It accounts for 9 per cent of total food grain production in the country. Karnataka, Rajasthan, Andhra Pradesh, Maharashtra, and Uttar Pradesh are the major maize producing states together contribute 60 per cent of area and 70 per cent of maize production in India. Last year in India in 2015-16, maize occupied 86.27 lakh ha area and production was estimated 13 per cent low about 210.2 lakh tonnes (Third Advance Estimates dt. 9-5-2016) as against 92.71 lakh ha and 241.7 lakh tonnes in previous year respectively. In Gujarat, the maize occupies more area in 2015-16 about 4 lakh ha as against 3.82 lakh ha in 2014-15 but production remains slight low about 5.96 lakh tonnes as against 6.31 lakh tonnes in previous year. Hence, maize price remain stable around Rs. 300 per 20 kg throughout the year with slight ups and downs. This is mainly due to reduction of export from 28.26 lakh tonnes in 2014-15 to about 6.5 lakh tonnes in 2015-16 as maize price remained slight higher than the world level.

Maize is ranks second to wheat among the world cereal crops (FAO 1986). Some 70 countries grow maize over 1,00,000 hectares or more, out of which 53 from the developing nations. Developed market economies account for 30% of the global maize area, but provide 50% of the total production as their average yields is three times higher than the world average. Developing nations accounted for 60% of the world total maize area, but produce only 40% of the global harvest **(Timothy et al., 1988).** In 1996, the total area under maize production was 141,116,000 ha^{-1} with a total yield of 590,091,000 metric tons of which 266,214,000 tons were harvested for grain.

MATERIAL AND METHOD

The experiment was conducted at research farm Chandra Shekhar Azad University of Agriculture and Technology, Kanpur during the Kharif season 2015 and 2016. The maize verity Azad Uttam were taken for study with 10 treatment and 4 replication the initial characterstics of soil (initial stage) were also analyse to know the nutrient status of soil. The soil of experimental field is low in organic carbon, available

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N_2 and available Zn but medium in case of available P, K and available S. The pH and EC was soil in normal range. The pH EC and organic carbon are analyse by the method described by Jackson (1967). Available N_2 was determine by Alkaline permanganate method as described by Subbiah and Asija (1956). Available phosphorus was extracted with 0.5 M $NaHCO_3$ Olsen et al. (1954). The P was determine in extract by vandomolybdate yellow colour method Jackson (1967). The available K was determined by flame photometer. Available sulphur was determined by Chesnin and Yien (1950). Available zinc was estimated by atomic absorption spectrophotometer. The plant samples were also analysed for N P K, S and Zn. Nitrogen was determined by Kjeldal's method (Jackson 1967). Phosphoras was determined calorimetrically (Chapman and Pratt, 1961). Potassium was determined by flame photometric method. Sulphar was determined by Chesnin and Yien (1956). Zinc was determined by atomic absorption spectrophotometer.

Table 1. Yield % increased over control on grain and Straw yield of maize in both year

Treatments	Grain yield in q ha^{-1}				Straw yield in q ha^{-1}			
	2015-16	% Increase over control	2016-17	% Increase over control	2015-16	% Increase over control	2016-17	% Increase over control
T1	14.33		14.85		38.91		39.48	
T2	21.16	32.16	22.30	33.40	56.52	31.33	57.39	31.20
T3	25.15	43.02	26.60	44.17	66.40	41.40	67.11	41.17
T4	24.40	41.27	25.60	41.99	66.29	41.30	67.76	41.73
T5	27.60	48.07	28.90	48.61	72.52	46.34	73.50	46.28
T6	24.92	42.49	26.96	44.91	67.97	42.75	68.43	42.30
T7	27.80	48.45	29.50	49.66	69.46	43.98	70.01	43.60
T8	27.15	47.21	28.40	47.71	68.64	43.31	69.66	43.32
T9	30.78	53.44	32.80	54.72	81.10	52.02	82.13	51.92
T10	26.16	45.22	29.40	49.48	69.63	44.11	68.87	42.67
S.E. (d)	**0.468**		**0.544**		**0.184**		**0.174**	
C.D. (P=0.05)	**0.960**		**1.116**		**0.378**		**0.358**	

Table 2. Effect of nutrients on plant population, plant height and test weight.

Treatments combination	No. of plants $plot^{-1}$*		Plant height (cm)		Test weight (100 grains)	
	2015-16	2015-16	2015-16	2016-17	2015-16	2016-17
Control	109.75	175.25	20.28	20.87	20.28	20.87
100 % NPK	110.75	180.75	23.58	24.11	23.58	24.11
100% NPK+S_{40}	111.00	182.50	25.30	25.72	25.30	25.72
100%NPK+Zn5	110.25	183.00	24.78	25.44	24.78	25.44
100%NPK+S_{40}+Zn5	110.75	190.00	28.03	28.53	28.03	28.53
125%NPK	110.25	189.00	24.65	25.22	24.65	25.22
125%NPK+S_{40}	111.50	194.00	27.25	27.75	27.25	27.75
125%NPK+Zn5	112.50	196.00	27.00	27.34	27.00	27.34
125%NPK+S_{40}+Zn5	112.25	213.75	32.37	33.01	32.37	33.01
150%NPK	110.25	192.25	27.92	25.75	27.92	25.75
S.E. (d)	**1.149**	**1.801**	**0.678**	**0.699**	**0.678**	**0.699**
C.D. (P=0.05)	**N.S.**	**3.696**	**3.589**	**N.S.**	**1.392**	**1.435**

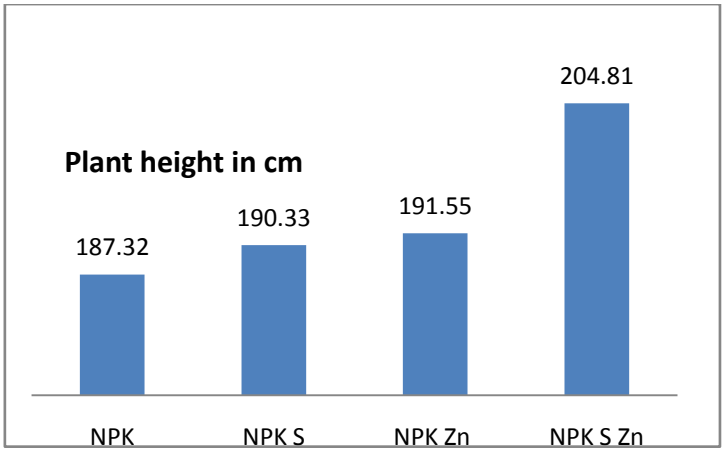

Fig. 1. Deferential responses of nutrients on plant height of maize

Table 3. Effect of nutrients on number of cobs and average length of cobs plant^{-1}

Treatments combination	number of cobs plant^{-1}		Mean	length of cob in (cm)		Mean
	2015-16	2016-17		2015-16	2016-17	
Control	1.00	1.10	1.05	13.40	14.60	14.00
100 % NPK	1.27	1.17	1.22	14.28	14.93	14.60
100% NPK+S_{40}	1.39	1.31	1.35	14.93	15.54	15.23
100%NPK+Zn5	1.38	1.30	1.34	14.69	15.24	14.96
100%NPK+S_{40}+Zn5	1.43	1.34	1.38	16.45	17.71	17.08
125%NPK	1.39	1.32	1.35	16.85	15.22	16.03
125%NPK+S_{40}	1.45	1.41	1.43	17.75	17.65	17.70
125%NPK+Zn5	1.44	1.40	1.42	16.83	17.47	17.15
125%NPK+S_{40}+Zn5	1.48	1.45	1.46	22.45	23.49	22.97
150%NPK	1.43	1.35	1.39	15.41	15.72	15.56
S.E. (d)	**0.020**	**0.044**		**0.345**	**0.422**	
C.D. (P=0.05)	**0.041**	**0.090**		**0.708**	**0.867**	

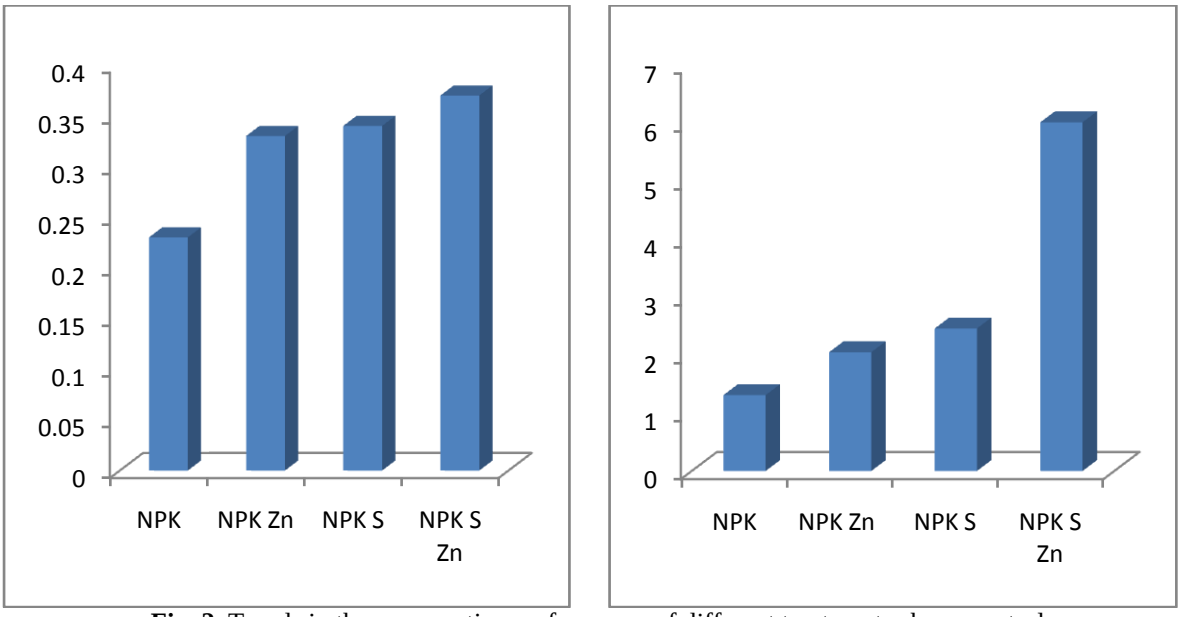

Fig. 2. Trends in the comparative performance of different treatments above control.

RESULT AND DISCUSSION

Fertilizers are play very important role in the yield of economy of the crop. Fertilizer alone contributed 55 to 60% to achieve the biological yield of a crop. In inceptisols five most limiting nutrients have been identified i.e. N,P,K,S and Zn. The element S and Zn are the recent additions in this list. The several side nutrient specific trails conducted at different locations both on farm and off farm established the

need of sulphur and zinc along with NPK for yield maximization. The results of present study are discussed as under:-

Grain yield:-. There were significant variations in the data under different treatments. During first year it varied from 14.33 to 30.78 q ha^{-1} with a mean value of 24.94 q ha^{-1}. About 53.44% yield increases with the addition of 125% N,P,K,S and Zn in comparison to control in first year and in during second year the data varied from 14.85 to 32.80 q ha^{-1} with a general mean value of 26.53 q ha^{-1}. About 54.72% yield increases with the addition of 125% N,P,K,S and Zn in comparison to control in second year. Several other scientists reported the results in conformity with the results of present study. Reddy et al.(2010), Zea et al. (2007) and Singh and Tripathi (2008).

Straw yield:- There were significant variations in the data under different treatments. During first year it varied from 38.91 to 81.10 q ha^{-1} with a mean value of 65.74 q ha^{-1}. About 52.02% yield increases with the addition of 125% N,P,K,S and Zn in comparison to control in first year during second year the data varied from 39.48 to 82.13 q ha^{-1} with a mean value of 66.43 q ha^{-1}. About 51.92% yield increases with the addition of 125% N,P,K,S and Zn in comparison to control. The dose of 125% NPK + S40+Zn5 gave the maximum straw yield. The results are statistically significant and all the treatment gave superior than control. Increased straw due to addition of N,P,K,S and Zn containing fertilizers has been reported by many workers Muthu Kumarraja et al. (2010). The results of present investigation are in agreement with these workers.

Plant population - It was observed that the treatment effect on plant population were not significant. However it varied from 109.75 to 112.50 and 109.60 to 111.67, during first and second years, respectively. The plant papulation of maize as affected by nutrient treatments is given in Table 1.

Plant height - As observed from the data all treatments has significantly greater plant height than control. It was further observed that 125% NPK was significantly superior to 100% NPK. Addition of sulphur to 100% NPK increased the plant height very near to significant value but did not reach the level of significance during both years. Addition of zinc to 125% NPK resulted in significant increase in plant height during both the years however the zinc had no significant effect with 100% NPK during both the years. The plant height of maize as affected by nutrient treatments is given in Table 1.

Test weight -The data revealed that test weight varied from 20.28 to 32.37 g and 20.87 to 33.01 g during first and second years, respectively. The treatment differences were significant and all the fertilizer treatments were significantly better than control. There was no significant difference in test weight under 100% and 125% NPK both the years but 150% NPK was significantly better than former two levels. Application of Sulphur, zinc and S+Zn proved significantly superior over 100% and 125% NPK alone. The test weight of maize as affected by nutrient treatments is given in Table 1.

Number of cob - The minimum and maximum numbers were recorded in control and 125%NPK+S_{40}+Zn5 and the latter came out to be the best treatment in respect of cob number during both the years. All the treatments were significantly superior to control. The number of cob of maize as affected by nutrient treatments is given in Table 2.

Cob length – Cob length was significantly affected by different treatments. It varied from 13.4 (in control) to 22.45 cm (in 125%NPK+S_{40}+Zn5) during first year and the same treatment offered range of variation from 14.6 to 23.48 cm. Hence 125%NPK+S_{40}+Zn5 was the best treatment in this respect and the next best treatment was 125%NPK+S_{40} with a mean value of 17.70 cm on the basis of mean of the years. Cob length was significantly increased due to application of 125% NPK over 100% NPK during first year but these levels did not differ significantly during second year. The cob length of maize as affected by nutrient treatments is given in Table 2.

CONCLUSION

The dose of 125% NPK+S40+Zn5 was the best dose amongs all in terms of grain yield, straw yield and yield attributing characters. So it is concluded that application of Sulphur and Zinc along with the combination of 125NPK gave best results to the farmers.

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RESEARCH ARTICLE

EFFECT OF INORGANIC NUTRIENTS AND BIO-INOCULANTS ON BLACKGRAM (*VIGNA MUNGO* L.)

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Abstract: A pot experiment on blackgram crop was conducted at pot house of the Department Soil Science and Agriculture Chemistry, C.S.Azad University of Agriculture and Technology Kanpur during kharif -2013 with variety shekhar-2 . The dose of experiment were 50% SR , 50% SR+Rh , 50% SR+PSB, 50%SR+Rh+PSB , 100% SR , 100 %SR+Rh , 100% SR+PSB, 100%SR+Rh+PSB , . The result showed that number of branches /plant varied from 1.5 to 4.5 and 2.5 to 5.5 at 30 and 60 DAS, respectively. The number of nodules ranged from 8.75 to 23.0 and 16.0 t0 30.50 at 30 and 60 DAS, respectively .The grain yield varied from 8.50 to 15.20 q/ha and stover yield varied from 12.60 to 23.80 q/ha . The N content in grain ranged from 3.16 to 4.24 % and P from 0.60 to 0.69 % .The N content in stover varied from 1.03 to 1.09 % and P From 0.24 to 0.29 % . The total nitrogen uptake ranged from 39.83 to 90.60 kg/ha and P uptake from 8.35 to 16.6 kg/ha . The protein content in black gram grain showing the range of variation from 19.75 to 26.62 % The treatment T_9 (100%SR+Rh+PSB) gave the best results in terms of branches , number of nodules, garain and stover yield, nutrient content, uptake values and protein content.

Keywords: Black gram, Crop, Inorganic nutrient, Production

INTRODUCTION

Being rich in protein, pulses not only form a vital part of the human diet, but also play a crucial role in balancing the dietary proteins. India holds the first rank in pulses production and consumption in the world. India grows the largest varieties of pulses in the world accounting for about 32% of the area and 23%of the world production. The important pulse crops are chickpea (48%), pigeon pea (16%), *urdbean* (9%), *mungbean* (7%), l entil (6%) and field pea (4%). The major pulse producing states are Madhya Pradesh (24%), Maharashtra (15%), Uttar Pradesh (12%), Rajasthan (12%) and Andhra Pradesh (9%), which together account for 72% of the total production. An estimated amount of 30 to 147 kg/ha biological nitrogen is fixed by different pulse crops in the soils in which they are grown. All data based on the book "State of Agriculture 2012-13" Government of India Ministry of Agriculture Department of Agriculture & Cooperation New Delhi.

Pulses production has registered a remarkable increase from 14.76 million tonnes in 2007-08 to a record level of 18.24 million tonnes in 2010-11. The production of pulses is estimated marginally lower at 17.09 million tonnes in 2011- 12. The increase in total production of pulses has been on account of improvement in production levels of *tur, urad* and *moong*. The average annual growth rate of area Bengal. However, the average productivity of pulses in India is less than the average productivity of 890 Kg/ha in world. Among the major pulses producing countries, the highest average yield of pulses has been recorded at 4219 Kg/ha in France, followed by 1936 Kg/ha in Canada and 1882 Kg/ha in USA in 2010. Cultivation of pulses is mostly (85% of the area) under rainfed condition, on Marginal lands , on low fertile soil by resource poor farmers. Non availability of High Yielding Variety, low Seed Replacement Rate (SRR), high susceptibility to pests especially *Helicover-pa armigera, inadequate* market linkage are the primary reasons for low yield of pulses. With a view to minimize the problem and ensure protective irrigation at the critical stage of plant development sprinkler set, mobile rain gun, pump set, etc. are distributed to farmers for efficient use of water from Dug well, Pond and Polythene lining pond. Further, the seed multiplication ratio (SRR) has been increased to 22.51% in 2010-11 from 10.41% in 2006-07.

To provide proper market infrastructure, the market linked extension support through Small Farmers Agribusiness Consortium (SFAC) under 60000 Pulses village programme is being implemented. Moreover, the Minimum Support Prices (MSPs) of Pulses have been increased substantially to incentivize Farmers to increase the production and productivity of pulses. Research Institutes like ICAR, IIPR, SAUs besides ICARDA and ICRISAT are making efforts to evolve varieties resistant to Helicoverpa. Emphasis is also being given on area expansion through promoting pulses cultivation in rice fallows, intercropping of pulses with oilseeds, cotton, cereals etc. Productivity enhancementthrough A3P demonstrations, INM,IPM& popularization/ promotion of the High Yielding varieties/hybrids. Under the National Food Security Mission (NFSM) from Rabi 2007-08, Accelerate Pulses Production Programme (A3P) is being implemented To accelerate The production of Pulses, particularly Red gram, Green gram, Blackgram, Chick pea and Lentil by promoting production and protection technologies. Integrated Development of 60000 Pulse Villages is implemented in selected watershed areas in major pulses growing states by providing

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funds for in-situ moisture conservation, new farm ponds with polythene lining and or dug wells. The special plan to achieve 19+ Million Tonnes of Pulses production is also under implementation during Kharif 2012-13.

METHOD AND MATERIAL

The experiment was conducted in Micro plots at pot culture of the size of 1ft × 1ft with the capacity of 20 kg in *"Pot culture house"* of the Department of Soil Science and Agricultural Chemistry, C.S. Azad University of Agriculture and Technology, Kanpur during Kharif season 2013. The soil of experimental pot low in organic carbon, available nitrogen and phosphorus but medium in case of potassium nutrient . The pH and EC of soil was in normal range. The soil was sandy loam in texture. The pH, EC and organic carbon are analyse by the method described by Jackson (1967). Available N_2 was determine by Alkaline permanganate method as described by Subbiah and Asija (1956). Available phosphorus was extracted with 0.5 M $NaHCO_3$ Olsen 0et al. (1954). The plant samples were also analyse for N and P. Nitrogen was determined by Kjeldal's method (Jackson 1967). Phosphorus was determined calorimetrically (Chapman and Pratt,1961). For quality characterstics the protein content in grain was also determined by the method described by Mc Cready and Hassid (1943).

Table 1. Effect of different treatments on the number of branches and number of nodules in blackgram:

Treatments	No. of Branches		No. of Nodules	
	At 30 DAS	At 60 DAS	At 30 DAS	At 60 DAS
Control	1.50	2.50	8.75	16.00
50%SR	3.25	4.25	10.75	20.25
50%SR+Rh	3.50	4.50	18.25	28.50
SR+PSB	3.75	4.25	16.50	27.50
50%SR+Rh+PSB	4.00	4.75	20.0	29.51
100%SR	4.25	4.50	18.50	30.25
100%SR+Rh	4.50	5.00	22.50	36.75
100%SR+PSB	4.25	5.25	22.01	35.50
100%SR+Rh+PSB	4.75	5.50	23.00	38.50
SE(d)	0.704	0.687	1.568	1.204
CD at 5%	1.461	1.427	1.718	2.500

Table 2. Effect of different treatment on grain, stover yield and protein content of blackgram .

Treatment	Grain Yield (q ha-1)	Stover yield (q ha-1)	Protein (%)
Control	8.50	12.60	19.75
T_2 50%SR	10.60	16.20	25.56
50%SR+Rh	11.70	18.00	25.87
50%SR+PSB	11.60	17.80	25.62
50% SR+Rh+PSB	11.90	18.30	26.08
100%SR	13.10	19.70	24.21

100%SR+Rh	14.30	21.90	26.31
100% SR+PSB	14.10	21.20	26.48
100% SR+Rh+PSB	15.20	23.80	26.62
SE(d)	**2.873**	**2.626**	**0.80**
CD at 5%	**1.384**	**1.265**	**1.62**

Table 3. Effect of treatments on nitrogen uptake in blackgram (kg ha^{-1})

S.N	Treatments	Grain uptake ($kgha^{-1}$)	Stover uptake ($kgha^{-1}$)	Total uptake ($kgha^{-1}$)	Grain uptake ($kgha^{-1}$)	Stover uptake ($kgha^{-1}$)	Total uptake ($kgha^{-1}$)
1	**Control**	26.86	12.97	39.83	5.11	3.24	8.35
2	**50%SR**	43.35	16.68	60.03	6.57	4.05	10.62
3	**50%SR+Rh**	48.43	18.9	67.33	6.74	4.5	11.24
4	**50%SR+PSB**	47.56	18.69	66.25	7.42	4.45	11.87
5	**50%SR+Rh+PSB,**	49.79	20.88	70.67	7.73	4.94	12.67
6	**100%SR**	50.69	18.86	69.55	8.64	5.31	13.95
7	**100%SR+Rh**	60.2	20.88	81.08	9.58	5.91	15.49
8	**100%SR+PSB**	59.64	23.43	83.07	9.58	5.93	15.51
9	**100%SR+Rh+PSB**	64.44	25.94	90.60	9.7	6.9	16.6
	SE (d)	**1.09**	**1.09**	**5.26**	**0.59**	**0.412**	**0.91**
	CD at 5%	**2.24**	**2.24**	**10.8**	**1.21**	**0.847**	**2.04**

RESULT AND DISCUSSION

The number of branches per plant were recorded at 30 DAS and 60 DAS and presented in table number 1 and It varied from 1.50 to 4.57 and 2.5 to 5.5 at 30 and 60 DAS, respectively .All the treatments gave significantly more branches than control .The treatment combination T_9 (100%SR+Rh+ PSB) gave the maximum number of branches at both the stages of crop growth. The increase in branch numbers of black gram may be described to the joint role of NPK role of N P K and bio- inoculants. The present study fall in the line with findings of several investigators (Saleh *et.al.*2013),

The numbers of nodules were significantly affected by application of rhizobium and PSB at 30 DAS and 60 DAS in current investigation. The nodule numbers are ranged from 8.75 to 23.0 and 16 .00 to 38.50 at 30 and 60 DAS, respectively. The treatment combination T_9 **(**100%SR+Rh+PSB) was best in case of nodule numbers. The nodule numbers are significantly superior in comparison to control. The effect of different treatments on number of nodules was presented in table 1. The increase in nodule numbers of by using of inorganic fertilizers and biofertilizers have been reported by several scientists like Amba *et.al*. (2013), Gawande et.al. (2007) and Tiwari *et.al*. (2005)

The grain and stover yield varied from 8.50 to 15.20 q/ha and 12.60 to 23.80 q/ha respectively . The maximum grain and stover yield was obtained by treatment combination T_9 (100%SR+Rh+PSB). All treatments are significantly superior than control in respect of grain and stover yield of blackgram is due to balanced nutrition through inorganic and biofertilizers. The effect of different treatments on grain yield and stover were presented in table 2. Similar kind of result has been reported Ahmad et.al. (2013).

The protein content varied from 19.75 to 26.62 % in grain of blackgram . The treatment T_9 (100%SR+Rh+PSB) gave the maximum quantity of protein. It mean the 100% dose of NPK along with inoculation of rhizobium and PSB increase the maximum protein content. The effect of different treatments on protein was presented in table 2.

The uptake value of nutrients in grain and stover increased due to concentration of these nutrients and biological yield of grain and stover. It was recorded that total N uptake varied from 39.83 to 90.60 kg/ha and P uptake ranged from 8.35 to 16.6 kg/ha .The uptake value indicate the appropriate quantity of nutrients required for optimum yield in present study . The effects of different treatments on uptake

values were presented in table 3. Similar kind of result have been reported by Singh and Chauhan (2005)

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RESEARCH ARTICLE

STUDIES ON ANTIOXIDANT ACTIVITY IN PULP AND PEEL OF SAPOTA (*MANILKARA ZAPOTA* L.) FRUITS IN DIFFERENT STAGES OF RIPENING

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Abstract: Fruits are major source of antioxidant enzymes. So, in this study the antioxidant activity and its related enzymes have been discussed in the peel and pulp of sapota during the three stages of ripening i.e. mature, half ripe and full ripe. Activity of all antioxidant and its related enzymes viz. superoxide dismutase, ascorbate peroxidase, peroxidase and glutathione reductase decreased during ripening from mature to full ripe stage. Mature fruits have highest content of ascorbic acid and all antioxidant enzymes. Peel of the fruit had higher activity of all antioxidant and its related enzymes as compared to pulp.

Keywords: Sapota, Pulp, Ripening stages, Antioxidant enzymes

INTRODUCTION

Fruits constitute a commercially important and nutritionally indispensable food commodity for human beings. Fruits are generally not part of staple diet but are helpful as a part of diet for providing essential minerals and protection from various diseases. Sapota commonly known as chiku in hindi, is rich sources of various vitamins, minerals, fibres and has a high calorific value. This fruit is used as antipyretic (Ganguly *et al*., 2013), antioxidant (Kulkarni *et al*., 2007), antibacterial (Chanda & Nagani, 2010), antimicrobial activity (Osman *et al*., 2011) and also has analgesic effect (Jain *et al*. (2011). Even the latex of fruit is helpful for filling the tooth cavities. Sapota is rich in antioxidant, because of various enzymes such as high activity of superoxide dismutase, glutathione reductase, peroxidase and catalase which are helpful to increase the antioxidant activity. However, fruits are diverse in antioxidant composition and antioxidant activity and those with high antioxidant activity generally contain more antioxidants (Guo *et al*., 1997). Sapota is one of the fruit which is consumed with peel and without peel. As peel of sapota is not having astringent value so it can be consumed along with peel. It is very well reported in literature that peel of fruit is having higher antioxidant activity, ascorbic acid and various antioxidant enzymes than pulp. However, there is no studies available in literature about these value in peel as well as pulp at various stages of ripening. So, present study was undertaken to compare the various antioxidant and its related enzymes at different stages of ripening in pulp and peel.

MATERIAL AND METHOD

Present experiment was conducted in the laboratory of department of Botany & Plant Physiology, CCS, Haryana Agricultural University, Hisar. The experiment was designed in completely randomized design. Fully mature fruits of sapota were harvested from the orchard of department of Horticulture, CCS, Haryana Agricultural University, Hisar with the secateurs keeping small intact pedicel with each fruits. Fruits were harvested and divided into 3 lots. Sapota fruits were categorized into mature, half ripe (50%) and fully ripe. The peels of Sapota fruits of uniform thickness were removed by fruit peeler from the pulp part and both pulp and peel were used for biochemical estimation separately. Ascorbic acid was determined by the titration method of AOAC (1990). The antioxidant activity of the fruit (peel and pulp) extracts in all three stages was evaluated by DPPH free radical scavenging method according to the method of Shimada *et al.* (1988). The activity of superoxide dismutase was assayed by the procedure of Beauchamp & Fridovich (1971). Ascorbate peroxidase was assayed by the procedure of Nakano & Asada (1981). Peroxidase was assayed according to the method described by Dias & Costa (1983). Glutathione reductase was analysed by the method of Halliwell & Foyer (1978).

RESULT AND DISCUSSION

Ascorbic acid content decreased with the ripening stages from 17.84 mg/100g in mature to 8.60 mg/100g in full ripe stage, when considered irrespective of peel and pulp. The reduction in ascorbic acid content in fruits may be due to oxidation of ascorbic acid into dehydro-ascorbic acid by ascorbic acid oxidase enzyme (Nayak *et al*., 2011). These results of decrease in ascorbic acid are in conformity with the previous findings of Iloki *et al.* (2013) in Noni (*Morinda citrifolia* L.) and Kamol *et al*. (2014) in pineapple. Peel of fruits at all three stages had higher content of ascorbic acid i.e. 19.27 mg/100g, 15.10 mg/100g and 9.77 mg/100g as compared to pulp of fruits i.e. 16.4 mg/100g, 13.44 mg/100g and 7.42 mg/100g in mature, half ripe and

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full ripe fruits respectively. The higher content of ascorbic acid in peel perhaps might have protected the fruit from infection of micro-flora from outer surface. These results are in agreement with the earlier reports of Abdrabba & Hussein (2015) in red Grape. Less ascorbic acid content indicated that the peel of the fruits should be consumed along with the pulp for antioxidant activity. Higher activity was observed in mature fruits i.e. 17.84 mg/100g whereas minimum in full ripe fruits i.e. 8.60 mg/100g. This indicates that for maximum antioxidant activity, fruits should be consumed in mature stage. However, mature fruits are not in good in taste. So, it is better that fruits should be consumed in half ripe stage.

Table 1. Ascorbic acid (mg/100g) and free radical scavenging activity (%) in Sapota fruit parts during different stages of ripening

Stages (S)	Ascorbic acid (mg/100g)			Free radical scavenging activity (%)		
	Fruit parts (P)			Fruit parts (P)		
	Pulp	Peel	Mean	Pulp	Peel	Mean
Mature	16.40	19.27	**17.84**	61.77	99.15	**80.46**
Half ripe	13.44	15.10	**14.27**	49.25	93.60	**71.43**
Full ripe	7.42	9.77	**8.60**	22.63	67.13	**44.88**
Mean	**12.42**	**14.72**		**44.55**	**86.63**	
CD at 5 %	**S=0.28 P=0.23 S×P=0.39**			**S=1.26 P=1.03 S×P=1.79**		

Free radical scavenging activity measured the efficiency of fruits to remove the free radical formed within the fruit. Higher is the activity of free radical scavenging activity more antioxidant it will be. Free radical scavenging activity decreased with the ripening stages from mature (80.46%) to full ripe stage (44.88%), when considered irrespective of peel and pulp. This might be due to high ascorbic acid present in fruits which might have been responsible for the higher activity of free radical scavenging activity. As there was direct correlation between ascorbic acid and free radical scavenging activity in half ripe and full ripe fruits also. As in half ripe and full ripe stage the content of ascorbic acid decreased which might be correlated with decreased in activity of free radical scavenging activity. This decrease in free radical scavenging activity results are also in conformity with the results of Iloki *et al.* (2013) in Noni (*Morinda citrifolia* L.). Peel of fruits at all three stages had higher content of free radical scavenging activity i.e. 99.15%, 93.60% and 67.13% as compared to pulp of fruits i.e. 61.77%, 49.25% and 22.63% in mature, half ripe and full ripe fruits respectively. This may be because ascorbic acid content was more in peel part of the fruit (Someya *et al*., 2002). The decrease in free radical scavenging activity results are in conformity with the results of Barros *et al*. (2012) in Citrus, and Woo *et al*. (2013) in Sapodilla.

Table 2. Superoxide dismutase activity (nmol/g fw) and ascorbate peroxidase activity (nmol/min/g fw) in Sapota fruit parts during different stages of ripening

Stages (S)	Superoxide dismutase (nmol/g fw)			Ascorbate peroxidase (nmol/min/g fw)		
	Fruit parts (P)			Fruit parts (P)		
	Pulp	Peel	Mean	Pulp	Peel	Mean
Mature	458.30	706.13	**582.22**	680.90	986.48	**833.69**
Half ripe	280.84	460.59	**370.72**	365.09	733.58	**549.34**
Full ripe	255.58	420.32	**337.95**	350.76	694.34	**522.55**
Mean	**331.57**	**529.01**		**465.58**	**804.80**	
CD at 5 %	**S=0.96 P=0.78 S×P=1.35**			**S=2.60 P=2.12 S×P=3.67**		

Superoxide dismutase (SOD) enzyme is a metaloprotein that catalyzes the dismutation of superoxide to H_2O_2 and molecular oxygen (Allen, 1995) (Table 2). It has been assumed that SOD had a central role in defense system against oxidative stress (Alscher *et al*., 2002). The SOD activity decreased from mature stage (582.22 nmol) to full ripe (337.95 nmol) fruit during ripening. This may be due to the reason that defense system decreases with the process of ripening. Similar decrease in SOD activity during ripening has also been reported in Guava (Goyal, 2010). The activity of enzyme has higher in the peel of the fruits 529.01 nmol as compared to 331.57 nmol in the pulp of fruits. Higher activity of SOD in the peel again indicates that peel is having better defense system than the pulp. Similar results of higher activity of SOD in peel of the fruit have also been reported by Abbasi *et al*. (2010) in 'Pink Lady' Apple fruit.

Table 3. Peroxidase activity (nmol/min/g fw) and glutathione reductase acitivity (nmol/min/g/ fw) in Sapota fruit parts during different stages of ripening

Stages (S)	Peroxidase (nmol/min/g fw)			Gtutathione reductase (nmol/min/g fw)		
	Fruit parts (P)			Fruit parts (P)		
	Pulp	Peel	Mean	Pulp	Peel	Mean
Mature	317.57	523.28	420.43	423.83	623.27	**523.55**
Half ripe	242.59	366.36	304.48	283.57	541.56	**412.57**
Full ripe	179.82	230.82	205.32	192.89	392.75	**292.82**
Mean	246.66	373.49		**300.10**	**519.20**	
CD at 5 %	**S=0.70 P=0.57 S×P0.99**			**S=0.78 P=0.63 S×P=1.10**		

Ascorbate peroxidase (APX) plays a pivotal role in eliminating hydrogen peroxide from plant cell (Madhusudhan *et al*., 2003). The activity of APX (Table 2) was found to decrease from 833.69 nmol in mature fruits to 522.55 nmol in full ripe stage when considered irrespective of pulp and peel. Decrease in activity of ascorbate peroxidase during ripening may be due to the reason that production of H_2O_2 decreases during ripening. The decrease in APX activity during ripening may also be either due to substrate being limited or the enzyme being inactivated. Highest APX activity at mature green stage followed by a continuos decrease during ripening has also been reported in Guava (Ram, 2007). Peel of the fruits (804.8 nmol) had higher activity of APX than pulp (465.58 nmol). The higher activity of enzyme ascorbate peroxidase in the peel indicates that more amount of H_2O_2 is produced in the peel than pulp. Similar observation of higher activity of APX in the peel has also been reported by Lata *et al*. (2005) who observed ascorbate peroxidase activity in peel and pulp of Apple cv. Elise.

The activity of peroxidase enzyme during ripening of Sapota is presented in table 3. Peroxidase activity is responsible for destroying H_2O_2 produced in fruits. Higher is the activity of peroxidase enzyme better antioxidant present in it. Peroxidase activity decreased from 420.43 nmol in mature stage to 205.32 nmol in full ripe stage when considered irrespective of pulp and peel. This may be due to reason that metabolic activity decreased from mature to full ripe stage. So, less H_2O_2 produced and as a results of which the activity of peroxidise enzyme also decreased. Similar decrease of peroxidase during ripening has also been observed by Goyal (2010) in Guava, Praduman (2010) in Ber fruits. Peel of the fruits has higher activity of the peroxidase enzyme i.e. 373.49 nmol as compared to pulp (246.66 nmol). The less activity of peroxidase in the pulp indicates that less metabolic rate in tissue of the pulp.

Glutathione reducatase (GR) activity of Sapota fruit was highest at mature stage (523.55 nmol) and declined to fully ripe stage (292.82 nmol) (Table 3) when considered irrespective of pulp and peel. As already reported in peroxidase enzyme, GR enzyme is also responsible for destroying of H_2O_2. The activity of which decreased during the process of ripening. This may also be because of reason that during the process of ripening the rate of various metabolic processes decreased which reduced the activity of this enzyme also. Decrease in GR activity during ripening has also been observed in Guava (Mondal *et al*., 2009) and Pradumen (2010) in Ber. Peel of the fruit had higher activity of GR (519.20 nmol) as compared to pulp part (300.10 nmol). This again indicate that metabolic activity are faster in peel part than the pulp. Similar finding of higher GR activity in peel has also been reported by Lata *et al*. (2005) in cv. Elise of Apple fruit.

CONCLUSION

From above all the studies, it is clear that, the peel of fruit is a good source of antioxidant and its related enzymes which are really required. Among the different studies, mature fruit had a higher content of antioxidant and its related enzymes and their activity decreased with the process of ripening. However, because of astringent taste fruits are not fit for consumption at mature stage. So, it is concluded from the studies fruits of half ripe stage are fit for consumption.

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RESEARCH ARTICLE

EFFECT OF FOLIAR APPLICATION OF NUTRIENTS ON SOYBEAN

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Abstract: An experiment was conducted at BAU experimental farm (Kanke), Ranchi, Jharkhand during (Soybean) *Kharif* season 2015 on sandy loam soil with low organic carbon (4.10 gkg^{-1}) and available nitrogen (192.5 $kgha^{-1}$), moderately acidic (pH 5.1) in nature, medium potassium (128 $kgha^{-1}$), phosphorus (13.65 $kgha^{-1}$), boron (0.58 $mgkg^{-1}$), molybdenum (0.25 $mgkg^{-1}$) and zinc (0.60 $mgkg^{-1}$), with 9 treatments replicated thrice. Results revealed that the productivity of soybean was influenced by foliar application of nutrients. Among application of nutrients, RDF along with molybdenum 0.5% spray produced higher grain (1524 $kgha^{-1}$) and straw (2062 $kgha^{-1}$) yield, which was significantly higher than all other treatment but it was at par with RDF + zinc chealted 0.5% spray and RDF + 19:19:19 ($N:P_2O_5:K_2O$) 2% spray. However, foliar application of zinc chelated 0.5% spray along with RDF gave highest net return (22630 $Rs.ha^{-1}$) and benefit: cost ratio (1.19).

Keywords: Economics, Soybean, Foliar, Nutrient

INTRODUCTION

Soybean is worldwide grown important oilseed crop, because it has a wide range of geographical adaption. Soybean plays a major role in the world food trade. It constitutes about 42% area and 56% production of total oilseeds. Production of soybean in India at present time restricted mainly to Madhya Pradesh and alone it contributes about 67 and 56% in total area and production of soybean respectively and is called as "Soya state". It is also grown in Uttar Pradesh, Maharashtra and Gujarat. In Chhotanagpur region of Jharkhand it is grown as rainfed crop. In global Soybean production India's rank fifth and in acreage fourth, occupying 12.22 million ha area with production of 11.86 MT and productivity 971 kg/ha, whereas in Jharkhand soybean occupies 6000 ha area with production of 2400 ton and productivity 400 kg/ha (AICRPS, 2014) which is about 41% less than national average.

To meet out increasing demand to feed the ever growing population farmeruse more and more chemical fertilizer to increase productivity. Abundant use of chemical fertilizer degrade the soil physico-chemical properties consequently factor productivity declining. In order to avoid or minimize the severity of such condition, foliar application of nutrients is imperative and it is being widely practiced to correctnutritionaldeficiencies in plants, which is better than soil application with increased fertilizer use efficiency (Silberbush*et.al.*, 2002) and also eco-friendly (Abou-El-nour, 2002). Under rainfed condition when the moisture availability is scarce, the application of fertilizer as foliar spray resulted in efficient absorption and it is most economical way of fertilization to achieve quality produce and higher productivity, especially when sink competition for carbohydrates among plant organs take place, while nutrient uptake from the soil is restricted (Kannan, 1986 and Singh, 2007). Flower senescence and poor filling of pods are the major drawbacks in soybean, which can be managed through foliar application of nutrient. Several workers reported that foliar application of nutrient is most effective way to achieve higher yield of crop. Foliar application of 1% urea during pod development stage resulted in better yield of soybean (Ashour and Thalooth, 2003). Similarly, foliar applications of urea in chickpea before terminal drought, at 50% flowering increased yield and seed protein content (Jairo*et al.*, 2005). Foliar application of murate of potash (MOP) increased the yield of soybean (Kelly *et al.*, 2005). Foliar application of N:P:K improve the ability of the plant for synthesis, storage and translocation of nutrient in common bean **(**Rahman*et al.*, 2014). Foliar applicationof zinc (Zn) increases the yield and protein content of soybean (Hugar and Kurdikeri, 2000).

MATERIAL AND METHOD

A field experiment was conducted on representative medium land soil of Jharkhand plateau of Birsa Agricultural University farm, Kanke, Ranchi (23^0 17' N latitude, $85^0$10' E longitude and 625 m above the mean sea level) during the *Kharif* season of 2015. The experimental plots had assured irrigation facility coupled with uniform topography, good drainage and soil characteristics which is typical suitable for soybean cultivation. The experimental site was a typical medium land with good soil depth and drainage facility. The soil was red type which belongs to the red yellow- light-grey category association group representing the major soil order (*alfisols*) of Jharkhand state, with moderately acidic in reaction (pH 5.1), low in organic carbon (4.10 gkg^{-1}) and available N (192.5 $kgha^{-1}$), medium in available phosphorus (13.65 $kgha^{-1}$), available potassium (128$kgha^{-1}$), available boron (0.58 $mgkg^{-1}$), available molybdenum (0.25 $mgkg^{-1}$) and available zinc (0.60

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mgkg^{-1}).The experiment was laid out in Randomized Block Design with nine treatments replicated thrice. Treatment comprised ofT_1- RDF + water spray, T_2- RDF + urea 2% spray, T_3- RDF + DAP 2% spray, T_4- RDF + MOP 0.5% spray, T_5- RDF + 19:19:19 ($N:P_2O_5:K_2O$) 2% spray, T_6- RDF + molybdenum 0.5% spray, T_7- RDF + boron 0.5% spray, T_8- RDF + zinc chelated 0.5% spray and T_9- RDF only.Here recommended dose of fertilizer (RDF)- 20:60:40:30 ($N:P_2O_5:K_2O:S$) and all the spray was given at pod initiation stage

The experimental soybean (**JS-97-52**) crop was sown on 15th July 2015 using seeds75 kg ha^{-1}.

RESULT AND DISCUSSION

Yield attributing characters and yield

Examination of the data revealed that the foliar application of nutrients significantly influenced the number of branches per plant. The maximum number of branches per plant (2.33) was recorded in RDF + molybdenum 0.5% spray, which was at par with RDF + zinc chelated 0.5% spray (2.30), RDF + 19:19:19 ($N:P_2O_5:K_2O$) 2% spray (2.29), RDF + urea 2% spray (2.25), RDF + DAP 2% spray (2.23), RDF + boron 0.5% spray (2.13) and RDF + MOP 0.5% spray. Maximum number of pods plant^{-1} (31) recorded with application of 0.5% molybdenum spray along with RDF and zinc chelated 0.5% spray along with RDF, which was significantly higher than all other treatment except RDF + 19:19:19 ($N:P_2O_5:K_2O$) 2% spray (30). Foliar application of molybdenum 0.5% spray, zinc chelated 0.5% spray, 19:19:19 ($N:P_2O_5:K_2O$) 2% spray along with RDF produced 63.15%, 63.15% and 57.89% higher number of pods plant^{-1} than control (RDF only) respectively. Analysis of data revealed that the different treatment failed to cause significant variation in number of seeds pod^{-1} and were non-significant. The highest number of seeds pod^{-1} (2.2) was recorded with application of RDF + 19:19:19 ($N:P_2O_5:K_2O$), RDF + molybdenum 0.5% spray and RDF + zinc chelated 0.5% spray while the lowest was with RDF alone (2.00). Among different foliar application, RDF + molybdenum 0.5% spray recorded maximum 100 seed weight (8.29 g) which was significantly higher than all other treatment except RDF + zinc chelated 0.5% spray (8.27 g), RDF + 19:19:19 ($N:P_20_5:K_2O$) 2% spray (8.21 g) and RDF + urea 2% spray (7.84 g). The minimum number of branches plant^{-1} (2.02), number of pods plant^{-1}(19), number of seeds pod^{-1} (2.00) and 100 seed weight (7.42 g) was recorded with RDF only.

Table 1. Effect of foliar application on yield attributes of soybean

Treatment	Yield attributes			
	Branches plant^{-1}	Pods plant^{-1}	Seeds pod^{-1}	100 seed weight (g)
RDF + Water spray	2.07	22.00	2.10	7.54
RDF + Urea 2% spray	2.25	27.00	2.10	7.84
RDF +DAP 2% spray	2.23	26.00	2.10	7.59
RDF + MOP 0.5% spray	2.12	24.00	2.10	7.70
RDF + 19: 19:19 ($N:P_2O_5:K_2O$) 2% spray	2.29	30.00	2.20	8.21
RDF + Molybdenum 0.5% spray	2.33	31.00	2.20	8.29
RDF + Boron 0.5% spray	2.13	25.00	2.10	7.83
RDF + Zinc chelated 0.5% spray	2.30	31.00	2.20	8.27
RDF only	2.02	19.00	2.00	7.42
SEm±	**0.07**	**1.04**	**0.11**	**0.18**
CD (P= 0.05)	**0.21**	**3.13**	**NS**	**0.54**
CV (%)	**5.57**	**6.94**	**8.68**	**3.98**

It is evident from the data (Table 2) that soybean crop fertilized with RDF along with molybdenum 0.5 % spray produced significantly higher grain yield (1524 kgha^{-1}) than all other combination of nutrients except RDF + zinc chelated 0.5% spray (1435 kg/ha) and RDF + 19:19:19 ($N:P_2O_5:K_2O$) 2% spray (1365 kgha^{-1}). The application of molybdenum 0.5% spray, zinc chelated 0.5% spray and 19:19:19 ($N:P_2O_5:K_2O$) 2% spray along with RDF brought 67.66%, 57.86% and 50.16% increase in grain yield of soybean over control (RDF only) and application of RDF along with molybdenum 0.5% spray produced significantly higher straw yield (2062 kgha^{-1}) than all other treatment except RDF + zinc chelated 0.5% spray (2010 kgha^{-1}). The straw yield of soybean increased by 42.80% and 39.20% when crop fertilized with RDF and sprayed with molybdenum 0.5% and zinc chelated 0.5% respectively over control (RDF only).The lowest grain yield (909 kgha^{-1}) and straw yield (1444 kgha^{-1}) was recorded in RDF alone.It is apparent from the data on harvest index revealed that the different treatment failed to cause significant variation in the harvest index and were non-significant. The highest harvest index was obtained with application of RDF+19:19:19 ($N:P_2O_5:K_2O$) 2% spray (42.81 %) while the lowest harvest index was recorded with RDF alone (38.66 %).

Yield increased with the increase in the number of branches plant^{-1}, pods plant^{-1} and 100 seed weight due to molybdenum (Mo) has a profound effect on plant reproductive development and seed yield (Kaiser *et al.,* 2005). Zinc (Zn) increase the

photosynthetic activity and delay the senescence of leaves, which enhances the supply of photosynthate available for grain filling thus resulted in bigger grains and ultimately yield will be increased (Tiwari*et al.,* 2011). Similar finding was reported by Sary*et al.,* 2014;Babaeian*et al.,* 2012 and Elankavi *et al.* (2009).

Table 2. Effect of foliar application on yield of soybean

Treatment	Yield		Harvest index (%)
	Grain yield ($kgha^{-1}$)	Straw yield ($kgha^{-1}$)	
RDF + Water spray	1102	1602	40.86
RDF + Urea 2% spray	1259	1818	40.75
RDF +DAP 2% spray	1233	1722	41.85
RDF + MOP 0.5% spray	1102	1648	40.07
RDF + 19: 19:19 ($N:P_2O_5:K_2O$) 2% spray	1365	1824	42.81
RDF + Molybdenum 0.5% spray	1524	2062	42.50
RDF + Boron 0.5% spray	1207	1721	41.37
RDF + Zinc chelated 0.5% spray	1435	2010	41.64
RDF only	909	1444	38.66
SEm±	**54**	**77**	**1.64**
CD (P= 0.05)	**163**	**229**	**4.91**
CV (%)	**7.62**	**7.51**	**6.89**

Economics of soybean

Data pertaining (Table 3) to cost of cultivation revealed that higher cost of cultivation was obtained with RDF + molybdenum 0.5% spray (23675 Rs ha^{-1}) while the minimum cost of cultivation (18550 Rs ha^{-1}) was found in control (RDF only).Soybean crop fertilized with RDF and sprayed with molybdenum 0.5% generate maximum and significantly higher gross return (45716 Rs ha^{-1}) than all other combination of nutrient except RDF + zinc chelated 0.5% spray (43055 Rs ha^{-1})and RDF + 19:19:19 ($N:P_2O_5:K_2O$) 2% spray (40952 Rs ha^{-1}).Minimum gross return (27268 Rs ha^{-1}) was recorded with control (RDF only). The gross return of soybean increased by 67.65%, 57.89% and 50.18% when crop fertilized with RDF and sprayed with molybdenum 0.5% spray, zinc chelated 0.5% spray and 19:19:19 ($N:P_2O_5:K_2O$) 2% spray respectively over control (RDF only). Among different treatment of nutrition, maximum net return (22630 `/ha) was recorded with application of zinc chelated along with RDF which remained at par with RDF + molybdenum 0.5% spray (22041 Rs ha^{-1}) RDF + urea 2% spray (18712 Rs ha^{-1}), RDF + 19:19:19 ($N:P_2O_5:K_2O$) 2% spray (18627 Rs ha^{-1}).The lowest net return (8718 Rs ha^{-1}) was obtained with control (RDF only). The application of zinc chelated 0.5% spray, molybdenum 0.5% spray, urea 2% spray and 19:19:19 ($N:P_2O_5:K_2O$) 2% spray along with RDF produced 159.58%, 152.82%, 114.63% and 113.65% higher net return over control (RDF only).

Table 3. Effect of foliar application on economics of soybean

Treatments	Cost of cultivation (Rs ha^{-1})	Gross return (Rs ha^{-1})	Net return (Rs ha^{-1})	B: C ratio
RDF + Water spray	18925	33055	14130	0.84
RDF + Urea 2% spray	19065	37777	18712	1.07
RDF +DAP 2% spray	19465	36990	17525	0.99
RDF + MOP 0.5% spray	19015	33055	14040	0.83
RDF + 19: 19:19 ($N:P_2O_5:K_2O$) 2% spray	22325	40952	18627	0.91
RDF + Molybdenum 0.5% spray	23675	45716	22041	1.00
RDF + Boron 0.5% spray	21425	36203	14778	0.77
RDF + Zinc chelated 0.5% spray	20425	43055	22630	1.19
RDF only	18550	27268	8718	0.56
SEm±	**-**	**1633**	**1633**	**0.08**
CD (P= 0.05)	**-**	**4896**	**4896**	**0.25**
CV (%)	**-**	**7.62**	**16.84**	**16.04**

Scrutiny of the data revealed that benefit: cost ratio of soybean was significantly influenced by the foliar application of nutrients. The maximum benefit: cost ratio (1.19) was observed with application of RDF + zinc chelated 0.5% spray which was significantly superior over all the other combination except RDF + urea 2% spray (1.07), RDF + molybdenum 0.5% spray (1.00) and RDF + DAP 2% spray (0.99). The lowest benefit: cost ratio (0.56) was recorded with control (RDF only) similar results reported by Kuttimani and Velayutham, 2011 and Zakaria*et al.,* 2008.

CONCLUSION

Foliar application of molybdenum 0.5% spray along with RDF produced higher yield attributes, grain yield as well as straw yield of soybean BUT foliar application of zinc chelated 0.5% spray along with RDF to soybean crop found most advantageous as it

produced highest net return and benefit cost ratio making it economically viable for the farmers in upland situation of Jharkhand. Based on the result of present investigation, it may be concluded that foliar application of zinc chelated 0.5% spray along with RDF proved to be more productive and economically viable for soybean cultivation.

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RESEARCH ARTICLE

CHARACTERIZATION AND CLASSIFICATION OF SOILS OF JHALARAPATAN BLOCK, JHALAWAR DISTRICT OF RAJASTHAN

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Abstract: A detailed soil survey of cluster of 10 villages in Jhalarapatan block, Jhalawar district of Rajasthan was carried out at 1: 8000 scales. Five typifying pedons representing undulating and alluvial plain landforms were studied for their morphological and physico-chemical properties. The soils were shallow to very deep, well to moderately well drained, moderately eroded, clay in texture developed over sandstone and basaltic parent materials. Soils of the undulating belong to *Typic Haplustept* while soils of the plain were classified as *Typic Haplustert*. The soils were slightly alkaline to strongly alkaline in reaction pH (7.78-8.76). Electrical conductivity ranged between 0.10-2.65 dSm^{-1}, organic carbon varied from 0.08-0.99 g kg^{-1}, cation exchange capacity ranged from 13.0- 56.5 cmol (p^{+}) kg^{-1}. Soils were low in available nitrogen (68.0-184.7 kg ha^{-1}), low to medium in phosphorus (3.4-20.2 kg ha^{-1}) and high in available potassium (1092 kg ha^{-1}). Soils were medium to high in available zinc (0.18-3.6 mg kg^{-1}), high in available iron (5.84-26.46 mg kg^{-1}) and copper (0.67-4.32 mg kg^{-1}). Available manganese was low to high (1.24-21.86 mg kg^{-1}).

Keywords: Soil, Survey, Morphology, Land resources

INTRODUCTION

Precise scientific information on characteristics, potentials, limitations and management needs of different soils is indispensable for planned development of land resources to maintain the soil productivity and to meet the demands of the future. Soil resource inventory provides an insight into the potentialities and limitations of soils for the optimum utilization. It also provides adequate information in terms of landform, terrain, vegetation as well as characteristics of soils which can be utilized for land resources management and development (Manchanda *et al.* 2002). Agricultural intensification and massive infrastructure development in recent years without considering the variability of entire production system enhances the risk of soil erosion and fertility depletion (Singh *et al.* 2007). In order to perform good management practices and remedial measures for various soil limitations, a systematic study of the soils is highly essential. Hence, the present investigation was taken up to characterize and classify the soils.

MATERIAL AND METHOD

Geographically, the Jhalarapatan block lies between $76^{o}10'30''$ E longitude and 24^{o} $32'10''$ N latitude. The general elevation of the study area also in ranges from 540 m above mean sea level (MSL). The drainage is essentially, subparallel and dendritic in the block. Climate of the block is sub- humid with mean annual rainfall of 833 mm, mean annual temperature of 25^{0} C. The relative humidity is 15-20 per cent. The area qualifies '*hyperthermic*' soil temperature regimes. Geology of the study area is dominantly of basaltic rocks, sandstone and shales with a band of limestone. Major part of the block is in the basin of Kalisindh and Ahu rivers. The major crops in the area are wheat, mustard, coriander, garlic, kalongi and gram in *Rabi* season and Jowar, maize cotton and soyabean in *Kharif* season.

The soil survey was carried out in ten villages covering an area of 2704.71 ha using base map at 1:8000 scale. A detailed traverse of the area was made to identify the landforms. Pedon sites were located in transects along the slope from the upper to lower slopes. Five typifying pedons were exposed and studied for morphological characteristics as per Soil Survey Manual (Soil Survey Division Staff, 1999). The horizon-wise soil samples were collected, air dried and passed through 2 mm sieve and analyzed for particle-size distribution following International Pipette method (Richards, 1954), pH and electrical conductivity (EC) in 1:2.5, soil : water suspension (Pipper 1966). Organic carbon was estimated by Walkley and Black (1934) method and calcium carbonate by rapid titration method (Piper 1966). The cation exchange capacity (CEC) and exchangeable cations were determined as described by Jackson (1958). The soils were classified as per Soil Taxonomy (Soil Survey Staff 2003).

RESULT AND DISCUSSION

Five pedons representing Neemoda-A, Neemoda-B, Khanpuriya-A, Khanpuriya-B and Salriya were described here under.

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Soil Morphology and Taxonomy

The data revealed (Table 1) that the soils of Neemoda-A series were deep, well drained, clay loam texture, very dark grayish brown (10YR 3/2) color and classified as Fine, smectitic, calcareous, hyperthermic *Typic Haplustert*. These soils occur on nearly level 1-3 per cent slopes whereas, the soils of Neemoda-B series were moderately deep, well drained, silty clay surface texture and clay sub-surface texture, very dark grayish brown (10YR 3/2) color and classified as Fine, mixed, calcareous, hyperthermic, *Typic Haplustept* which are developed on sandstone and basaltic materials. The soils of Khanpuriya-A were shallow, well drained, silty loam surface texture, dark brown (10YR 3/3) to very dark grayish brown (10YR 3/2) in colour and classified as Fine-silty, mixed, calcareous hyperthermic *Typic Haplustept*, whereas, the soils of Khanpuriya-B series were moderately shallow, well drained, silty clay surface texture followed by silty clay sub-surface texture, dark brown (10YR 3/3) to very dark grayish brown (10YR 3/2) in color and classified as Fine silty-over cleyey, mixed, hyperthermic, *Fluventic Haplustept*. The soils of Salariya series were very deep, well drained, clay texture, dark brown (7.5YR 3/2) in color and classified as Very-fine, smectitic calcareous, hyperthermic *Typic Haplusttept*.

The color of soils under moist condition from pedon I to II was very dark grayish brown (10YR 3/2), whereas, the color of pedon III to IV was dark brown (10YR 3/3) and color of pedon V was dark brown (7.5YR 3/2). This difference in colour may be attributed to the organic matter and high clay content (Diwakar and Singh 1992).

The structure of the soils was sub-angular blocky with variable grades. The relative distribution of cations like Ca^{2+}, fe^{2+} etc. along with organic matter appear to be influencing the development of mostly angular blocky structures (Diwakar and Singh 1992; Diwakar 2005). The consistency of these soils was hard, moderate sticky and moderately plastic, under dry and moist conditions. It might be attributed to the dominance of smectite of clay minerals along with the clayey texture of the soils (Diwakar and Singh 1992).

Physico-chemical properties of soils

The data (Table 2) indicate that the sand content in soils ranged from 2.0 to 59.7 per cent with a mean value of 18.6 per cent. Higher sand content is noticed in soils of Khanpuriya-A and Khanpuriya-B developed on plain landforms. The silt content ranged from 14.9 to 73.2 per cent with a mean value of 41.3 per cent. The silt content in all the pedons have irregular trend with the depth. Clay content ranged from 16.2 to 74.2 per cent with a mean value of 40.1per cent. Higher clay content is noticed in soils of Neemoda-B and Salariya. Higher clay content was found in the sub-surface horizons because of the illuviation of fine fractions from the surface layers.

Soils of Neemoda-A series are slightly alkaline to moderately alkaline pH (7.78 to 8.36), (Table 2) whereas, the soils of Neemoda-B and Khanpuriya-A series are moderately alkaline to strongly alkaline pH (7.90-8.58) and the soils of Khanpuriya-B and Salariya series are strongly alkaline pH (8.02-8.64). The electrical conductivity (EC) ranged from 0.10 to 2.65 dSm^{-1}. Except two soil samples all are under < 1 dSm^{-1}. It indicates that they are non-saline in nature as suggested by Muhr *et al.* (1963) comparatively low content of soluble salts appear to be due to the type of climate (sub-humid) of the area which is fairly sufficient to leach out major part of soluble salts from the soil. The organic carbon (OC) content in these soils is ranged from 0.08 to 0.99 g kg^{-1}. It showed a considerable variation with types and topography of soil. Relatively higher values of organic carbon can be ascribed to annual addition of plant residues and also the application of FYM.

Cation exchange capacity of typifying pedons ranged from 13.0 to 56.5 cmol (p^+) kg^{-1} with an average value of 29.37 cmol (p^+) kg^{-1}. The CEC increased with increase in clay content of the pedons. Higher values of CEC in sub-surface horizon commensurate with amount of clay .The CEC increased with depth in the pedons of Khanpuriya-A and Salariya series due to increase in clay content of lower horizons. The CEC decreased with depth in the pedons Neemoda-A, Neemoda-B and Khanpuriya-B series due to variation in clay and organic matter content (Mishra and Ghosh, 1995). The soils of Neemoda-A, Neemoda-B, Khanpuriya-A and Salariya series are calcareous whereas the soils of Khanpuriya-B are non-calcareous. The exchangeable bases had distinct pattern regarding their sequential dominance. In all the pedons, the order followed was Ca>Mg>Na>K. Similar results were observed by Diwakar and Singh (1994). The Ca^{2+} in soils ranged from 4.34 to 39.4 cmol (p^+) kg^{-1}, with a mean value of 19.83 cmol (p^+) kg^{-1}, Mg^{2+} ranged from 1.2 to 20.2 cmol (p^+) kg^{-1} with a mean value of 11.8 cmol (p^+) kg^{-1}, Na^+ ranged from 0.01 to 1.5 cmol (p^+) kg^{-1}, with a mean value of 0.17 cmol (p^+) kg^{-1} and K^+ ranged from 0.02 to 0.24 cmol (p^+) kg^{-1} with a mean value of 0.08 cmol (p^+) kg^{-1} (Table 3.).

Fertility status

The fertility status of soils revealed (Table 3) that available nitrogen was low (68.0-184.7 kg ha^{-1}), phosphorus was low to medium (3.4-20.2 kg ha^{-1}) and available potassium was high (1092 kg ha^{-1}). Available zinc content ranged from 0.18-3.6 mg kg^{-1} mg kg^{-1}. Considering 0.6 mg kg^{-1} as the critical limit given by Singh *et al.* (2003), Soils were in general medium to high in available zinc. Available copper ranged between 0.67-4.32 mg kg^{-1}. Considering 0.4 mg kg^{-1} as the critical limit given by Singh *et al.* (2003), all the samples were found sufficient to adequate in available copper. Available iron in soils

ranged from 5.84-26.46 mg kg^{-1}. Based on the critical limit as suggested by Lindsay and Norvell (1978) is 4.5 mg kg^{-1} for iron the soils were high in available iron. It may be due to the presence of iron bearing minerals in the soil. Available manganese ranged from 1.24-21.86 mg kg^{-1}. Singh *et al*, (2003) suggested 4.0 mg kg^{-1} as critical limit and accordingly the sufficient amount of Mn in soils was due to the presence of Mn containing minerals.

CONCLUSION

From the present study it can be concluded that distinct relationship exists between soil properties and landform units. The soils on different landforms are at varying degree of pedogenic development. Major part of the block is under Kalisindh and Ahu rivers Predominant influence of Kalisindh and Ahu river system was found on the soil formation and landscape which modified the basic soil characters. The major crops in the area are wheat, mustard, coriander, garlic, kalongi and gram in *Rabi* season and Jowar, maize cotton and soya bean in *Kharif* season. Orange is an important crop in the block,which is a major source of income for farmers. The soil resource data generated in the present investigation could be well utilized for general crop planning of the area with site specific management practices. The data generated will provide the characteristics of soils and fertility status in a map of a particular area so that any scientist, researcher, planner and farmer can utilize the data for further planning. However, more study is needed on pedogenic development of these soils.

Table 1. Morphological characteristics of typifying pedons of Jhalarapatan Block.

Horizon	Depth (cm)	Colour (moist)	Texture	Structure	Gravels	Effervescence	Root distribution
Pedon 1 (Neemoda-A): Fine, smectitic, calcareous, hyperthermic Typic Haplustert							
Ap	0-23	10YR 3/2,	cl	massive	5-10	e	fm
Bss1	23-30	10YR 3/2,	c	m2sbk	5-10	e	fm
Bss2	30-50	10YR 3/2,	c	m2sbk	>10	e s	mm
BC	50-73	10YR 3/4,	l	m2sbk	>35	ev	mm
Ck	73-120	Weathered rock	l	-	>50	ev	-
Pedon 2 (Neemoda-B): Fine, mixed, calcareous, hyperthermic, Typic Haplustept							
Ap	0-18	10YR 3/2	sic	m1sbk	15	e	fm
Bw1	18-35	10YR 3/2	c	m2sbk	-	e	cf
Bw2	35-52	10YR 3/2	c	m2sbk	-	e	cf
Bk	52-90	10YR 4/2	cl	m2sbk	60	ev	-
R	90+	10YR 4/2	-	-	-	ev	-
Pedon 3 (Khanpuriya-A) **:** Fine-silty, mixed, calcareous hyperthermic Typic Haplustept							
Ap	0-9	10YR 3/3	sil	m1sbk	-	-	mvf
Bw1	9-26	10YR 3/2	sil	m2sbk	-	-	cvf
Bw2	26-36	10YR 3/2	sil	m2sbk	-	es	cf
Ck	36-50	10YR 5/2	scl	-	-	ev	-
Pedon 4 (Khanpuriya-B) **:** Fine silty-over cleyey, mixed, hyperthermic, Fluventic Haplustept							
Ap	0-9	10YR 3/3	sil	m1sbk	<10	-	vf
Bw1	9-18	10YR 3/2	sil	m1sbk	<10	-	cf
Bw2	18-37	10YR 3/2	c	m1sbk	<10	-	cf
II Ck	37-60	10YR 5/2	scl		>50	e	-
Pedon 5 (Salariya): Very-fine, smectitic calcareous, hyperthermic Typic Haplusttept							
Ap	0-15	7.5YR 3/2	c	massive	-	es	ff
Bss1	15-45	7.5YR 3/2	c	m2sbk	-	e	ff
Bss2	45-70	7.5YR 3/2	c	m2sbk	-	e	-
Bss3	70-100	5YR 3/4	c	m2sbk	-	-	-
Bss4	100-170	2.5YR 3/4	c		-	-	-

Table 2. Physico-chemical properties of typifying pedons of Jhalarapatan block.

Horizons	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	pH	EC (dSm^{-1})	OC (g kg^{-1})	$CaCO_3$ (g kg^{-1})	CEC [cmol (p^+) kg^{-1}]	Exchangeable cations			
										Ca	Mg	Na	K
										cmol (p^+) kg^{-1}			
Pedon 1 (Neemoda-A): Fine, smectitic, calcareous, hyperthermic Typic Haplustert													
Ap	0-23	22.60	40.68	36.72	7.78	0.71	0.99	6.61	29.0	14.68	14.0	0.08	0.24
Bss1	23-30	15.39	37.99	46.62	8.01	0.44	0.35	5.05	32.4	20.05	12.2	0.09	0.06
Bss2	30-50	15.46	35.60	48.94	8.05	0.45	0.31	5.69	34.4	18.82	15.4	0.13	0.05
BC	50-73	38.80	35.32	25.88	8.06	0.49	0.19	12.69	19.2	5.42	13.6	0.15	0.03
Ck	73-120	40.02	43.79	16.19	8.36	0.32	0.16	16.66	13.0	5.16	18.0	0.14	0.02
Pedon 2 (Neemoda-B): Fine, mixed, calcareous, hyperthermic, Typic Haplustept													
Ap	0-18	8.54	48.64	42.82	8.52	0.38	0.84	5.94	31.0	27.72	3.2	0.01	0.07
Bw1	18-35	9.74	39.61	50.65	8.67	0.32	0.52	7.05	36.1	31.04	5.0	0.11	0.06
Bw2	35-52	9.73	39.30	50.95	7.90	0.27	0.52	9.74	36.7	34.3	2.2	0.15	0.05
Bk	52-90	39.32	31.25	29.43	8.76	0.45	0.19	21.66	21.8	18.0	3.0	0.77	0.03
R	90+	-	-	-	-	-	-	-	-	-	-	-	-
Pedon 3 (Khanpuriya-A) **:** Fine silty-mixed, calcareous hyperthermic Typic Haplustept													

Ap	0-9	9.01	73.19	17.8	8.30	0.37	0.42	4.35	14.32	13.0	1.2	0.08	0.04
Bw1	9-26	10.17	72.27	17.56	8.38	0.30	0.39	4.61	14.8	13.0	1.7	0.07	0.03
Bw2	26-36	11.52	64.70	23.78	8.52	0.34	0.40	9.61	17.2	16.0	1.1	0.07	0.03
Ck	36-50	49.27	22.05	28.68	8.58	0.22	0.11	23.32	21.2	12.49	8.6	0.07	0.04
Pedon 4 (Khanpuriya-B) **:** Fine silty-over cleyey, mixed, hyperthermic, Fluventic Haplusttept													
Ap	0-9	7.28	67.82	24.90	8.53	0.11	0.41	3.50	17.8	12.0	5.7	0.06	0.04
Bw1	9-18	8.19	69.05	22.76	8.54	0.10	0.25	3.78	16.25	12.0	4.2	0.03	0.02
Bw2	18-37	13.12	31.64	55.24	8.42	0.11	0.20	4.06	38.4	20.33	18.0	0.05	0.02
II Ck	37-60	59.7	14.9	25.40	8.53	0.16	0.08	5.56	19.2	4.34	14.6	0.04	0.02
Pedon 5 (Salariya): Very fine-smectitic calcareous, hyperthermic Typic Haplustept													
Ap	0-15	14.37	31.75	53.88	8.41	0.25	0.55	4.50	37.3	21.36	12.2	0.03	0.24
Bss1	15-45	13.07	27.43	59.57	8.64	0.23	0.41	4.35	41.4	25.46	15.6	0.13	0.21
Bss2	45-70	9.41	26.36	64.23	8.47	0.37	0.22	4.18	48.5	39.04	9.0	0.23	0.23
Bss3	70-100	2.94	31.44	65.62	8.07	2.08	0.18	3.68	49.8	36.35	12.8	0.44	0.21
Bss4	100-170	2.04	23.76	74.20	8.02	2.65	0.17	2.95	56.5	35.82	19.0	1.5	0.18

Table 3. Range and mean values of physico-chemical properties and nutrients of soils of Jhalarapatan block.

Ranges	Soil properties																		
	Sand	Silt	clay	pH	EC	OC	$CaCO_3$	CEC	Ca	Mg	Na	K	N	P	K	Cu	Zn	Mn	Fe
	(%)	(%)	(%)		(dSm^{-1})	$g\ k^{-1}$	$g\ k^{-1}$	cmol (p+) kg^{-1}	cmol (p+) kg^{-1}				Kg ha^{-1}			mg kg^{-1}			
Min.	2.0	14.9	16.2	7.78	0.10	0.08	2.95	13.0	4.34	1.2	0.01	0.02	68.0	3.4	98.6	0.66	0.18	1.24	5.84
Max.	59.7	73.2	74.2	8.76	2.65	0.99	23.32	56.5	39.4	20.2	1.50	0.24	184.7	20.2	1092.0	4.32	3.60	21.86	26.46
Mean	18.6	41.3	40.1	8.34	0.50	0.35	7.70	29.37	19.83	11.8	0.17	0.08	129.7	9.1	394.5	2.39	0.57	7.38	11.41

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RESEARCH ARTICLE

GENETIC VARIABILITY STUDIES FOR YIELD, OIL AND MORPHO-PHYSIOLOGICAL TRAITS IN SOYBEAN (*GLYCINE MAX* (L.) MERRILL)

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Abstract: Thirty five different elite germplasm lines of soybean along with five checks were sown during kharif, 2011 in an experiment laid out at experimental farm of All India Coordinated Research Project on soybean, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani during kharif, 2011 with a view to study the genetic variability for yield, oil and morpho-physiological traits in soybean. The study revealed that the genotypes viz., EC 3412, EC 257303, MACS 609, JS 20-29, JS 93-05, MAUS 162, NRC 87, SL 778, MACS 1281, NRC 86, Swarna Vasundhara, VLS 77, MACS 1259, AMS-MB 5-19, Monetta and MACS 1201 exhibited better performance for number of branches per plant, number of pods per plant, 100 seed weight, harvest index, leaf area index, oil content. High genetic coefficient of variation was observed for seed yield per plant, number of pods per plant, number of branches per plant, leaf area index, plant height and harvest index. High heritability coupled with high expected genetic advance was observed for number of pods per plant, seed yield per plant, number of branches per plant and 100 seed weight. Hence, direct selection for these characters in soybean will increase the breeding efficiency. The promising genotypes viz., EC 3412, EC 287303, MAUS 609, JS 20-29, JS 93-05, MAUS 162, NRC 87, SL 778, MACS 1281, NRC 86, Swarna Vasundhara, VLS 77, MACS 1259, AMS-MB 5-19, Monetta, MACS 1201 should be further evaluated for yield and other characters in future.

Keywords: Genetic variability, Heritability, Soybean, Yield

INTRODUCTION

Soybean has emerged as one of the major oilseed crop in India with the coverage of above 10.12 million hectare with estimated production of over 10.22 million tonnes during 2011-12. In Maharashtra state, soybean crop is grown on an area of 30.6 lakh hectare with total production 38.45 million tonnes with average productivity of 1256 kg/ha(Anonymous, 2012). Soybean occupies the central Indian niche predominantly in Madhya Pradesh, Maharashtra and Rajasthan.

Crop improvement is based on magnitude of the genetic variability in the base material and extent of heritability of desirable characters (Dhillion *et al*., 2005). For improvement of crop yield, the breeder has to select superior individuals based on their phenotypic expression. Selection based on phenotypic expression is sometimes misleading as the development of the character may be due to the result of interaction of the heritable and non heritable factors. This highlights the imperative need for partitioning the overall variability into its heritable and non heritable components and genetic parameters like genetic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability and expected genetic advance (EGA). Knowledge of genetic variability and genetic advance is essential for a breeder to choose best genotype and to decide the correct breeding methodology for crop improvement. Therefore, the present study was carried out to study the genetic variability and genetic advance for yield, oil and morpho-physiological traits in soybean.

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MATERIAL AND METHOD

The experimental material for the present investigation comprised of 35 genotypes of soybean (*Glycine max* (L.) Merrill) collected from Directorate of Soybean Research, Indore and germplasm lines maintained at All India Coordinated Research Project on Soybean, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani. These germplasm lines were selected on the basis of their diverse geographical origin and variation in yielding ability.

Thirty five different elite germplasm lines of soybean along with five checks were sown during *kharif*, 2011 in an experiment laid out at experimental farm of All India Coordinated Research Project on Soybean, Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani on 15th July, 2011. The experiment was conducted in Randomized Block Design with two replications with 45 cm row to row distance, 5 cm plant to plant distance and 30:60:30 NPK (kg/ha) fertilizer dose was applied. All recommended agronomic package of practices and plant protection measures were followed for satisfactory crop growth. Five competitive plants were selected randomly from each treatment in each replication for recording of observations. Observations were recorded on morphological characters *viz.*, days to 50% flowering, days to maturity, plant height (cm) and number of branches per plant. Physiological traits *viz.*,leaf area index, chlorophyll content (mg) and harvest index (%) and yield contributing characters *viz.*, number of pods per plant, 100 seed weight (g), oil content (%), protein content (%) and seed yield per plant (g). The data

recorded on these characters was analyzed for the means and genetic variability. The genotypic and phenotypic variances were calculated by using the respective mean squares from variance table (Johnson *et al.*, 1955). The genotypic and phenotypic coefficient of variation (GCV) and (PCV) were calculated according to method suggested by Burton (1952).

RESULT AND DISCUSSION

Analysis of variance showed significant genetic variability for all the characters studied which is depicted in the table-1. The mean performance of 35 genotypes along with five checks for twelve characters is presented in table 2. Early maturity was recorded in the genotypes *viz.*, EC 3412 (82.0 days) and SL 871 (88.0 days). The genotypes *viz.*, EC 257303 (5.9), MACS 609 (5.7), PS 1476 (5.6), MACS 1281 (5.5), EC 3412 (5.0), SL 778 (5.0) and JS 20-29 (5.0) recorded maximum number of branches per plant. Number of pods per plant were found higher in the genotypes *viz.*, EC 257303 (53.4), MACS 609 (48.7), JS 20-29 (43.1), KDS 344 (41.90), NRC 86 (41.01) and JS 93-05 (42.2). Highest 100 seed weight were recorded in genotype Suwarna Vasundhara (26.0 g) indicating its bold size followed by SL 778 (18.0 g), and JS 20-29 (16.1 g). High oil content recorded in genotype Swarna Vasundhara (22.59 %) followed by DS 28-11 (22.23 %). The strains *viz*., Swarna Vasundhara (21.86 g), EC 25 7303 (19.09 g), JS 93-05 (17.50g), JS 20-29 (16.25g) and MACS 609 (15.49 g) recorded highest seed yield per plant. Highest protein content was recorded in genotype NRC 87 (42.83 %) followed by KS 103 (41.69 %) and NSO 81 (41.12 %) while highest LAI was recorded in genotype NSO 81 (2.51) followed by NRC 87 (2.30) and Suwarna Vasundhara (2.22).

Range of variability

Wide range of variability was observed for majority of yield contributing characters (Table-3). Range of variation on the basis of mean was found more for the traits *viz.*, number of pods per plant, plant height, harvest index, days to maturity, seed yield per plant, days to 50 per cent flowering, 100 seed weight, chlorophyll content, leaf area index, protein content, oil content and number of branches per plant. Similar trend of results were obtained by Dhillion *et al*. (2005) and Gupta and Punetha (2001).

Estimates for phenotypic variance were found higher than the genotypic variance for all of the characters (Table-3). High genotypic variances were observed for the character plant height followed by number of pods per plant, harvest index, days to maturity, seed yield per plant, days to 50 per cent flowering, leaf area index, chlorophyll content, 100 seed weight, protein content, oil content and number of branches per plant. High phenotypic variance was observed for plant height, number of pods per plant and days to maturity. These findings are in agreement with those results reported by Malik *et al.,* (2006) and Sirohi *et al.,* (2007).

Genotypic and phenotypic coefficient of variation

In the present investigation, the phenotypic coefficient of variation were greater than the genotypic coefficient of variation, but the difference between them were found to be at lower magnitude indicating that there is small effect of environment on the characters under study and selection may be effective for improvement of these traits.

The highest values of genotypic coefficient of variation and phenotypic coefficient of variation were recorded for the characters *viz.,* seed yield per plant, number of pods per plant, number of branches per plant, leaf area index and plant height indicating the possibilities of enhancement of these traits through selection. Similar observations were made by Karnwal and Singh (2009). Low estimates of phenotypic coefficient of variation and genotypic coefficient of variation was observed for the traits *viz.,* protein content, oil content, days to maturity, days to 50 per cent flowering and chlorophyll content. These results are in conformity with reports of earlier workers *viz.,* Sahay *et al*. (2005) and Karnwal and Singh (2009).

Heritability and genetic advance

The knowledge about heritability of a trait is helpful to enable the plant breeder for deciding appropriate selection procedure to be followed for improvement of trait under given situation. The high heritability estimates along with expected genetic advance is more useful in predicting yield under phenotypic selection than heritability estimates alone (Johnson *et al*., 1955).

In the present investigation range of heritability was from 35.40 per cent for days to maturity to 91.22 per cent for seed yield per plant. The desirable broad sense heritability (More than 60%) was observed for days to 50 per cent flowering (61.04 %), number of branches per plant (69.94 %), plant height (70.21 %), chlorophyll content (75.97 %), number of pods per plant (86.43 %), harvest index (78.91 %) and seed yield per plant (91.22 %). These results are in agreement with those results obtained by Dixit *et al.* (2002). Karnwal and Singh (2009) and Patil *et al.* (2011) reported high genetic advance for the traits *viz.,* number of pods per plant, seed yield per plant, number of branches per plant, plant height, 100 seed weight and leaf area index suggesting that these characters were governed by additive gene effects. Similar trend of results were found in the present investigation.

High heritability estimates coupled with high expected genetic advance were observed for the characters *viz.,* seed yield per plant, number of pods per plant, number of branches per plant and plant

height indicating the presence of additive gene action in expression of these characters. Similar results were reported by Sahay *et al*. (2005) and Karnwal and Singh (2009). In the present investigation, high heritability coupled with low genetic advance was observed in traits *viz.*, days to 50 per cent flowering, days to maturity, leaf area index, chlorophyll content, harvest index, oil and protein content. Similar type of results were reported by Sahay *et al*. (2005) and Karad and Kadam (2005) indicating the presence of poor genetic variance in the material for these characters.

Thus, from the foregoing discussion, it is clear that characters *viz.*, seed yield per plant, number of days to 50 per cent flowering, number of branches per plant, number of pods per plant, plant height, and harvest index recorded high heritability with high expected genetic advance indicating the presence of additive gene action and phenotypic selection will be effective for these traits while formulating a breeding programme.

Table 1. Analysis of Variance for the studied morphological, yield contributing and physiological traits in soybean

S. V.	D. F.	Mean Sum of Square											
		Morphological Characters					Yield Contributing Characters				Physiological Traits		
		Days to 50% Flowering	Days to Maturity	Plant Height (cm)	No. of branches/ plant	No. of pods/ plant	100 seed weight	Oil content	Protein content	Seed yield/ plant	Harvest index	Leaf area index	Chlorophyll content
1	2	3	4	5	6	7	8	9	10	11	12	13	14
Replication	1	7.200	14.450	52.035	0.084	53.284	0.276	0.635	0.003	0.159	10.138	0.003	0.075
Treatment	39	21.364**	69.200**	257.449**	1.428**	207.209**	14.481**	4.656**	11.718**	30.342**	101.038**	0.175**	0.152**
Error	39	5.174	32.014	45.046	0.252	39.444	4.197	1.659	4.452	1.39	11.907	0.040	0.020

Table 2. Mean performance of soybean genotypes for morphological, physiological and yield and yield contributing traits in soybean

Sr. No.	Genotype	Morphological traits				Physiological traits			Yield and yield contributing traits				
		Days to 50% flowering	Days to maturity	Plant height (cm)	No. of branches/ plant	Leaf Area Index	Chlorophyll Content (mg)	Harvest index (%)	No. of pods/ plant	100 seed weight (g)	Oil content (%)	Protein content (%)	Seed yield per plant (g)
		1	2	3	4	5	6	7	8	9	11	12	10
1	AMS-MB-5-19	39.50	100.50	55.34	4.5	1.84	3.37	47.59	40.04	11.65	21.53	36.22	10.69
2	PS 1480	38.50	105.50	64.43	3.8	1.17	2.95	42.16	21.91	13.25	17.11	37.46	7.71
3	JS(SH)2003-8	42.00	104.50	72.00	3.6	1.42	3.18	52.96	22.13	14.0	18.11	38.44	7.74
4	CSB-08-08	44.00	102.00	71.84	3.2	1.05	2.90	26.16	15.78	11.40	18.19	38.28	3.68
5	EC 3412	35.50	82.00	38.36	5.0	1.24	3.28	35.07	34.80	15.05	18.01	38.19	13.56
6	MAUS 162	45.50	98.50	81.13	4.3	1.37	3.31	49.52	33.40	14.55	18.21	35.48	14.01
7	KSO 245	38.50	100.50	53.14	3.6	1.99	3.22	45.03	27.30	13.85	17.86	34.82	6.58
8	VLS 77	38.00	105.50	55.78	4.1	1.78	3.37	33.55	34.32	15.50	19.35	36.13	13.27
9	AMS-MB-5-18	38.00	104.50	53.27	2.6	1.35	3.04	33.56	38.02	12.25	18.56	38.16	10.61
10	KS 103	38.00	103.00	72.11	4.5	1.73	3.27	45.57	32.95	12.25	17.09	41.69	7.93
11	DSB 18	41.00	103.00	75.99	3.6	1.98	3.23	39.42	25.30	13.80	18.19	42.83	7.53
12	NRC 87	40.50	97.50	46.99	4.1	2.27	4.16	49.74	33.10	13.90	19.06	39.13	12.48
13	CSB-08-09	48.50	106.50	80.44	2.3	2.00	3.50	29.54	14.50	9.95	17.13	39.28	3.67
14	SL 871	38.50	88.00	56.11	4.5	1.38	3.06	50.71	25.80	13.35	19.44	32.71	9.37
15	Swarna Vasundhara	40.50	98.00	57.09	4.7	2.16	3.79	56.81	29.70	26.00	22.59	33.47	21.86
16	Monetta	35.50	94.50	59.89	3.2	1.94	3.72	44.55	34.60	12.55	20.54	36.81	12.00
17	NRC 2008 F 1	36.50	98.00	48.53	4.3	1.55	3.16	42.87	27.10	12.20	20.50	40.55	8.55
18	PS 1476	40.00	102.50	66.10	5.6	1.32	3.10	47.44	24.70	13.40	19.23	34.52	9.36
19	JS 93-05	37.00	94.50	46.49	4.9	1.49	3.28	46.29	42.20	15.65	19.82	37.31	17.50
20	SL 778	35.50	95.00	61.27	5.0	1.48	3.33	56.68	33.80	18.00	19.99	32.53	15.18
21	RKS 61	42.00	95.00	55.00	3.1	1.58	3.16	43.97	26.00	9.35	18.79	40.47	5.09
22	MACS 1259	36.50	99.00	80.13	3.3	1.71	3.34	48.17	24.30	12.40	21.26	38.77	7.49
23	MACS 1311	37.50	98.00	58.07	4.3	1.55	3.25	41.50	36.80	14.55	21.50	35.37	10.54

24	DS 27-11	40.00	104.00	56.53	3.5	1.24	3.13	41.80	30.80	14.45	22.23	38.86	9.29
25	MACS 1281	41.50	100.00	71.11	5.5	1.47	3.15	49.52	40.40	13.55	21.09	34.35	11.45
26	Kalitur	42.50	105.00	81.36	4.2	1.90	3.40	39.94	30.10	12.35	19.79	39.10	7.45
27	NSO 81	35.50	102.00	56.98	3.5	2.20	3.75	42.74	20.05	12.75	20.66	41.12	7.70
28	EC 257203	39.50	103.00	50.30	5.9	1.47	3.44	57.00	53.40	15.35	21.51	37.93	19.09
29	MACS 1201	42.50	98.50	62.31	3.7	1.84	3.47	49.39	26.50	15.60	20.80	34.34	10.14
30	KDS 344	43.00	104.50	69.01	3.2	1.55	3.34	39.99	41.90	10.60	19.01	37.27	9.74
31	NRC 86	35.50	89.50	48.95	4.3	1.78	3.67	45.02	41.50	12.65	21.76	33.58	11.35
32	Bragg	42.00	102.50	56.83	4.7	1.56	3.40	49.99	37.95	12.45	21.17	35.15	11.02
33	JS 20-29	37.00	93.50	59.74	5.0	1.32	3.39	50.07	43.10	16.10	20.43	37.05	16.25
34	VLS 76	33.00	105.50	48.96	2.9	1.57	3.17	53.97	22.20	14.35	21.29	36.26	6.78
35	MAUS 609	38.00	96.00	54.97	4.3	1.61	3.66	51.01	48.70	15.25	21.59	36.56	15.49
	CHECKS												
36	MAUS 47	36.50	82.50	41.07	4.3	1.66	3.46	41.44	30.20	14.00	18.45	37.88	10.35
37	MAUS 71	38.00	98.00	49.05	4.0	1.27	3.15	52.10	30.40	11.90	20.22	38.80	10.62
38	MAUS 158	40.00	94.50	62.71	4.7	1.75	3.62	46.75	40.30	12.95	19.79	37.34	12.32
39	JS 335	37.50	97.50	53.17	4.7	2.00	3.73	50.07	38.00	14.45	20.36	36.18	12.34
40	JS 97-52	44.00	99.50	68.02	4.0	1.70	3.59	49.42	42.60	9.75	21.87	38.21	9.92
	G. mean	39.35	98.80	60.13	4.14	1.63	3.37	45.48	32.47	13.69	19.85	37.21	10.69
	SE +	1.60	4.06	4.74	0.35	0.14	0.10	2.44	2.34	1.44	0.91	1.49	0.83
	CD at 5%	4.57	11.54	13.49	1.01	0.40	0.28	6.93	6.65	4.11	2.58	4.24	2.37
	CD at 1%	6.09	15.39	17.98	1.34	0.53	0.38	9.24	8.87	5.49	3.45	5.65	3.16

Table 3. Parameters of genetic variability for morphological, physiological and yield and yield contributing traits in soybean.

Sr. No.	Character	Range	Mean	Genotypic variance (σ^2g)	Phenotypic variance (σ^2p)	GCV (%)	PCV (%)	Heritability (%)	Expected genetic advance (%)
1	Days to 50% flowering	33.00-48.5	39.35	8.09	13.26	7.23	9.25	61.04	11.63
2	Days to maturity	82-106.5	98.80	18.09	51.10	4.30	7.23	35.40	5.27
3	Plant height (cm)	38.36-81.36	60.13	106.20	151.24	17.13	20.45	70.21	29.58
4	Number of branches per plant	2.3-5.9	4.14	0.58	0.84	18.48	22.10	69.94	31.85
5	Number of pods per plant	14.5-53.4	32.47	69.89	80.86	25.74	27.68	86.43	49.30
6	100 seed weight (g)	9.35-26.00	13.69	5.14	9.33	16.56	22.32	55.05	25.31
7	Leaf area index	1.17-2.27	1.63	6.74	10.76	15.89	20.07	62.65	25.91
8	Chlorophyll content (mg)	2.90-4.16	3.37	6.57	8.64	7.60	8.72	75.97	13.65
9	Harvest index (%)	26.16-57.00	45.48	44.56	56.47	14.67	16.52	78.91	26.86
10	Oil content (%)	17.09-22.59	19.85	1.49	3.15	6.16	8.95	47.45	8.7
11	Protein content (%)	32.53-42.83	37.21	3.63	8.08	5.12	7.64	44.93	7.07
12	Seed yield per plant (g)	3.67-21.86	10.69	14.47	15.86	35.57	37.24	91.22	69.99

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RESEARCH ARTICLE

ESTIMATES OF VARIABILITY PARAMETERS FOR YIELD AND ITS COMPONENTS IN SOYBEAN (*GLYCINE MAX* L.)

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Abstract: The present study of genetic variability was carried out using 30 genotypes of soybean for 8 quantitative characters. Analysis of variance for the design of experiments indicated highly significances among treatments for all the characters. Wide range of variation was found for seed yield per plant, plant height, 100-seed weight, number of pods per plant , number of secondary branches per plant , number of primary branches per plant ,number of seeds per pod, indicated good scope for improvement. Maximum phenotypic and genotypic coefficients of variation were observed for plant height followed by number of primary branches per plant, seed yield per plant, number of clusters per plant, number of pods per plant and pod length.

Keywords: Soybean, Variability, Heritability, Yield

INTRODUCTION

Soybean (*Glycine max* (L.) Merrill) is one of the important oil yielding crop of India. It has nutritional, medicinal and industrial uses. India is the fifth largest producer of Soybean and sixth in Soybean oil in the world. Soybean occupies an area of about 7.172 million hectare with an annual production of 12.2 million tons and average productivity of 1140 kg/hectare in India (USDA, 2013). In Madhya Pradesh, it is grown in an area of 59.062 Lakh hectare with a production of 34.125 Lac tones with productivity of 608 kg/ha (SOPA, 2015). Seed yield per hectare of this crop is low in India. Its cultivation under poor crop management and non-availability of quality seed of improved soybean varieties are the major reasons for low productivity of the crop. Thus, there is need to develop or identify high yielding Soybean varieties. Development of high yielding cultivars requires information on nature and magnitude of variation in the available germplasm. The observed variability is a combined estimate of genetic and environmental cause of which only the former one is heritable. Heritability and genetic advance of the seed yield and its components is pre-requisite for the improvement through selection. The present investigation provides better insight and scope for the improvement of seed yield through component characters in Soybean.

MATERIAL AND METHOD

The experiment material comprised 30 Soybean strains/varieties were grown in Randomized Block Design with three replications at Research Farm, Rajoula, Mahatma Gandhi Chitrakoot Gramodaya Vishwavidyalaya, Chitrakoot, Satna (Madhya Pradesh) during *Kharif* 2014. Observations were recorded on five randomly selected plants from each plot for Eight quantitative characters *viz.* days to flowering, days to 50% flowering, plant height (cm.), number of primary branches per plant, number of secondary branches per plant, number of pods per plant, number of seeds per pods, 100-seed weight (gm.), and seed yield per plant (gm.). The variability parameters were determined as per the methodology suggested by Burton and de Vane (1953) and Johnson *et al.,* (1955).

RESULT AND DISCUSSION

Present study, the extent of variability was estimated in the 30 germplasm/ varieties of soybean for their 08 characters. Analysis of variance for various characters is given in Table -1. Design of the experiment indicated highly significant differences among the genotypes for all the eight characters under study which indicating the presence of high genetic variability in the materials. Wide range of variation was found for seed yield per plant, plant height, 100-seed weight, number of pods per plant , number of secondary branches per plant , number of primary branches per plant ,number of seeds per pod, indicated good scope for improvement. Mean, range, GCV, PCV, as per cent of mean are presented in Table -2. The variability estimates, in general, phenotypic coefficient of variation (PCV) was higher than corresponding genotypic coefficient of variation (GCV). The estimates of phenotypic and genotypic coefficients of variation indicated the existence of fairly high degree of variability for seed yield per plant, 100 seed weight and plant height. Moderate variability was observed for number of secondary branches per plant, and no pods per plant. The minimum genotypic and phenotypic coefficients of variation were observed for days to 50% flowering,

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Number of primary branches per plant and number of seeds per pod. Days to first flowering, days to 50% flowering, plant height, number of pods per plant, number of seeds per pod and 1000-seed weight showed almost similar values of phenotypic and genotypic coefficients of variation, indicating that variability was primarily was due to genotypic differences and environment has played little role in the expression of this character. The observations are in agreement with the findings of Savita *et al*. (2007) and Dubey *et al.* (2007).

The major function of heritability estimates is to provide information on transmission of characters from the parents to the progeny. Heritability and Genetic advance are presented in Table -3. Such estimates facilitate evaluation of hereditary and environmental effect in phenotypic variation and thus aid in selection. Heritability estimates are used to predict expected advance under selection so that breeders are able to anticipate improvement from different of selection intensity. Johnson *et al*. (1955) have suggested heritability estimates in association with genetic advance are much useful for selection than heritability alone.

In the present study, estimates of heritability in broad sense ranged from 30.00 per cent for days to 50% flowering to 98.70 for 100-seed weight. High heritability estimates was found for 100-seed weight followed by seed yield per plant, plant height, no. of primary branches, no. of seeds per pod while low estimates was found in no. of secondary branches and days to 50% flowering. It indicated less environmental influence on the characters and recorded high transmission index. The high heritability indicates that these traits are generally governed by the additive gene action and that the genotypes of the traits strongly reflect the phenotypes. Similar results were reported by Tewari *et al*. (1989), Borah and khan (2000), Nehru and Manjunath (2001), Pal *et al*. (2003), Nigude *et al.* (2004), Prasanthi (2004), Eswaran *et al*. (2007), Nehru *et al*. (2009) and Manggoe *et al*. (2012). Burton (1952),

The expected genetic advance in per cent of mean ranged from 3.49 per cent for days to 50% flowering to 48.05 per cent for 100-seed weight (Table 4.3). High estimates of expected genetic advance were found for 100-seed weight, seed yield per plant and plant height while moderate to low genetic advance was found for number of pods per plant , number of primary branches per plant , number of secondary branches per plant, number of seeds per pod and days to 50% flowering. Similar were the findings of), Borah and khan (2000), Pal *et al*. (2003), Nigude *et al.* (2004), Malik et al. (2006) for 100-seed weight, Eswaran *et al*. (2007), and Nehru *et al*. (2009) and Badkul *et al*. (2014)for no. of branches per plant andno. of pods per plant.

Table 1. Analysis of Variance for Eight quantitative characters in Soybean.

S. No.	Character	Mean Sum of Square		
		Replication	Treatments	Error
	d.f.	2	29	58
1	Days to 50% Flowering	3.68	9.12**	3.95
2	Plant Height(Cm.)	20.80	124.39***	18.39
3	No. of Primary Branches per plant	0.26	1.53***	0.43
4	Number of Secondary Branches	4.21***	0.51***	0.19
5	No.of Pods Per Plant	348.51***	26.12***	9.02
6	No. of Seeds Per pod	0.89	0.123***	0.04
7	100 Seed weight(g)	0.45**	14.77***	0.07
8	Seed yield per plant(g)	23.63***	7.57***	0.83

*** Significant at 5% probability level; **Significant at 1% level probability level.**

Table 2. Mean, range, genotypic and phenotypic coefficient of variation for 8 quantitative characters in Soybean

S. No.	Characters	Grand mean (`X) + SE (m)	Range		Component of variability		Coefficient of Variation	
					Genotypic	Phenotypic	GVC	PCV
			Min.	Max.				
1	Days to 50% Flowering	42.69 ±1.15	39.00	46.00	1.72	5.68	3.08	5.58
2	Plant Hight(Cm.)	25.8 ±2.48	14.68	39.97	35.33	53.73	22.96	28.31
3	No. of Primary Branches per plant	6.20 ± 0.38	4.80	7.80	0.37	0.80	9.75	14.40
4	No. of Secondary Branches	3.02± 0.25	2.20	4.20	0.11	0.30	10.79	18.12
5	No.of Pods Per Plant	21.25 ±1.73	14.47	27.07	5.70	14.72	11.24	18.05
6	Number of Seeds Per pod	2.82 ± 0.12	2.00	3.13	0.03	0.07	5.76	9.43
7	100 Seed weight(g)	9.43 ± 0.15	6.58	14.38	4.90	4.97	23.48	23.63
8	Seed yield per plant(g)	5.65± 0.53	2.77	8.89	2.25	3.08	26.53	31.06
8	Seed yield per plant(g)	5.65± 0.53	2.77	8.89	2.25	3.08	26.53	31.06

Table 3. Heritability (%) in broad sense, genetic advance and genetic advance in percent of mean for 08quantitative characters of Soybean

S.N.	Characters	Heritability (%)	Genetic advance	Genetic advance in percent of mean
1	Days to 50% Flowering	30.4	1.49	3.491
2	Plant height(Cm.)	65.8	9.93	38.347
3	No. of Primary Branches per plant	45.9	0.843	13.608
4	Number of Secondary Branches	35.4	0.399	13.235
5	No.of Pods Per Plant	38.7	3.061	14.403
6	No. of Seeds Per pod	37.3	0.204	7.245
7	100 Seed weight(g)	98.7	4.531	48.048
8	Seed yield per plant(g)	73	2.637	46.675

High heritability estimates coupled with high genetic advance were observed for seed yield per plant, harvest index, biological yield per plant, number of pods per plant, number of pods per cluster, number of cluster per plant and 1000-seed weight indicated that these traits were mostly controlled by additive gene action. Phenotypic selection for these characters would be highly effective as also reported earlier by Tewari *et al*. (1989),), Borah and khan (2000), Pal *et al*. (2003), Nigude *et al.* (2004), Eswaran *et al*. (2007), Nehru *et al*. (2009) and Sureshrao *et al*. (2014)for number of pod per plant, seed yield per hectare and seed yield per plant .

The present study revealed that the 100 seed weight, seed yield per plant and plant height possessing high heritability along with high genetic advance and high to moderate variability estimates indicating a greater scope for the improvement through selection from the population.

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RESEARCH ARTICLE

PLANT GROWTH REGULATORS AFFECTING SEX EXPRESSION OF BOTTLE GOURD [*LAGENARIA SICERARIA* (MOL.)] CV. PUSA SUMMER PROLIFIC LONG

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Abstract: The investigation was carried out in the experimental farm of Department of Horticulture, S.K.N Agriculture University, Jobner,Jaipur (Rajasthan) to see the effect of various plant growth regulators and thiourea on vegetative growth, sex expression, quality and yield attributes of bottle gourd cv. cv. Pusa Summer Prolific Long, during the season 2012. The experimental was laid out with 13 treatments in randomized block design and replicated thrice. The treatment comprised of plant growth regulators and thiourea, viz. T_0 (control), T_1 (100 ppm NAA), T_2 (200 ppm NAA), T_3 (300 ppm NAA), T_4 (150 ppm Ethrel), T_5 (300 ppm ethrel), T_6 (450 ppm ethrel), T_7 (100 ppm CCC), T_8 (200 ppm CCC), T_9 (300 ppm CCC), T_{10} (250 ppm thiourea), T_{11} (500 ppm thiourea), T_{12} (750 ppm thiourea). The results revealed that the application of NAA 300 ppm (T_3) recorded maximum vine length (6.80 m), nodes per vine(22.01) and leaf area (274.00 cm^2). The CCC 300 ppm (T_9) treatment produced maximum primary branches (22.97) and secondary branches (9.30) per vine and leaf area (203.26 m^2) were observed in this treatment. The results showed that NAA 300 ppm registered maximum vegetative growth, ethrel 750 ppm significantly decreased male flower (65.60). Most of the quality parameters are maximum at ethrel 450 ppm as crude protein contents (0.226), ascorbic acid (12.90), TSS (5.31%). It may be concluded that ethrel 400 ppm (T_6) was found most effective as it remained statistically at par in all the growth, flowering attributes and yield.

Keywords: Bottle guard, PGRs, Thiourea, Vegetative growth, Flowering, Yield, Quality

INTRODUCTION

Bottle gourd [*Lagenariasiceratia*(mol.) standl.] cv. Pusa summer prolific long is a commonly grown and used vegetable in India. It belongs to family cucurbitaceaes. Besides an important vegetable crop it has also got good medicinal as well as nutritional value. Due to these qualities and people now days becoming more health conscious. The intake of its demand has been increasing day by day. The fruits are also used as vegetable or for making sweets/halwa, kheer, petha, barfi and pickles. It is economically found growing in Ethiopia, Africa and Central America. In India, bottle gourd is grown in the area of 532.7 thousand hectares with annual production of 6346.4 thousand metric tones and having 11.9 metric tonnes/hectares productionvity (NHB Database, 2014). It occupies 5,120 hectares area in Rajasthan producing 17857 matric tones with a productivity of 3.4876 tonnes ha^{-1} (Anonymous, 2011). In Jaipur district is occupies 1164 hectares area producing 1419 matric tones with a productivity of cucurbits, the growth regulators are more important due to their direct effect on males and female flowers ratio, fruit set, fruit drop and ultimately on yield. The use of plant growth regulators at proper stage plays an important role in sex expression and yield of bottle gourd (Sircael, 1971). In bottle gourd, the production of staminate flowers is much more than pistillate flower. These situations lead to the use of plant growth regulators like NAA, Ethrel and CCC in bottle gourd which play an important role in sex expression and sex ratio and thiourea plays a vital role in the physiology of plants both as a sulphydryl compound and to some extent as an amino compound like urea. The stimulating action of thiourea in various physiological activities of plants is well known. Keeping these facts in view, the present investigation was under taken with objectives to know the effect of plants growth regulators and thiourea on growth yield and quality of bottle gourd.

MATERIAL AND METHOD

Field experiment was conducted on Bottle gourd cv. Pusa summer prolific long the experimental farm of the department of horticulture, S.K.N. College of Agriculture, Jobner, Jaipur (Rajasthan). The soil of experimental farm was sandy loam texture, having organic carbon 0.13%, pH 8.2 and EC0.85 (dSm^{-1}). The treatment comprised of plant growth regulators and thiourea viz. T_0 (control), T_1 (100 ppm NAA), T_2 (200 ppm NAA), T_3 (300 ppm NAA), T_4 (150 ppm Ethrel), T_5 (300 ppm ethrel), T_6 (450 ppm ethrel), T_7 (100 ppm CCC), T_8 (200 ppm CCC), T_9 (300 ppm CCC), T_{10} (250 ppm thiourea), T_{11} (500 ppm thiourea), T_{12} (750 ppm thiourea) were designed in randomized block design with replicated thrice. The gross plot size was 6m x 3m with spacing of 2.5 m x 0.75 m (row x plant). The bottle gourd three to flower seeds per pit was sown on 12th July 2012 by dibbling method. Bottle gourd crop was fertilized with recommended dose i.e. 250 FYM, 60, 40, 60 NPK kg ha^{-1}. Nitrogen, phosphorus and potash fertilizer were

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applied in the form of urea, single super phosphate and muriate of potash respectively. Half dose of nitrogen and whole quantity of phosphorus and potash were applied. The remaining half dose of nitrogen (60 kg/ha) was top dressed after one month of sowing. The observation on growth and yield parameters were recorded during investigation.

RESULT AND DISCUSSION

The data pertaining to the effect of plant growth regulators and thiourea on vine length are presented in Table 1. The data revealed that plant growth regulators and thiourea significantly increased as well as decreased the vine length and number of nodes per vine. The maximum vine length and number of nodes per vine was recorded under foliar spray of NAA 300 ppm (T_3) and minimum with CCC 300 ppm (T_9) but treatment NAA 200 ppm (T2) was found at par with NAA 300 ppm (T_3). Chhonkar and Singh (1959) assigned that the increase in vine length under application of NAA was on account of its stimulatory effect on obserption of available nutrient present in the soil or by the modification in plant root system through the associated microflora of the soil similar results were also reported by Das and Das (1996) in pumpkin due to application of NAA (150 ppm).

The increased nodes on vine axis under exogenous application of NAA 250 ppm might be due to stimulatory effect of NAA on vine growth, cell division, cell elongation, cell enlargement (Chhonkar and Singh, 1959). These findings are also in conformity with Randhawa and Singh (1976) in bottle gourd and Chovatia *et al.* (2010) in bitter gourd cv. Priya.

The maximum leaf area was found under exogenous application of NAA 300 ppm (T_3) and minimum in CCC 300 ppm (T_9) are presented in Table 1. The effect of NAA on leaf area might be due to physiological process of plant that leads to accumulation of carbohydrates and minerals, which showed that gibberellins and auxins have some role in leaves other than cell elongation. These finding are in close conformity with the results reported by Sharma et al. (1988) in bottle gourd, Kabir *et al.* (1989) in bitter gourd, Das and Maurya (1992) and Das and Das (1996) in pumpkin, respectively.

The results of the present investigation revealed that the maximum number of primary branches and secondary branches were found under CCC 300 ppm (T_9) and minimum in control respectively (Table-1). These results are also in accordance with Randhawa and Singh (1976) in bottle gourd.

Among different treatment NAA 300 ppm (T_3) showed minimum days for first male flower appearance as compared to control (T_0) are presented in Table 1. The results obtained are in agreement with those Baruas and Das (1997) in Bottle gourd, respectively.

The data regarding to the effect of plant growth regulators and thiourea on all the fruit quality attributes are depicted in Table 2. Ethrel 450 ppm contributed maximum crude protein content, ascorbic acid and TSS in fruits as compared to control. These results are consonance with the finding of Shafeek *et al.* (2016) in summer squash guard.

The maximum fruit yield per vine and per hectare under ethrel 450 ppm (T_6) might be due to higher number of female flower, higher number of fruits and higher fruit weight under ethrel 450 ppm (T_6) are presented to table 2. These finding are in close fonformity with those of Kumar *et al.* (2006) in bottle gourd, Das and Das (1996) in pumnpkin and Jadav *et al.* (2010) in cucumber.

Table 1. Impact of plant growth regulators and thiourea on vegetative growth and flowering parameters of bottle gourd [Lagenaria siceraria (Mol.) cv. Pusa Summer Prolific Long]

Treatment	Vegetative growth parameters					Flowering attributes	
	Vine length (m)	No. of nodes /vine	Leaf area (cm^2)	No. of primary branches/vine	No. of secondary branches/vine	No. of male flowers/vine	Days taken to first male flower appearance
T_0: Control	**4.50**	**20.12**	**210.40**	**8.01**	**4.05**	**95.17**	**58.12**
T_1: NAA 100 ppm	6.80	24.42	260.10	13.40	6.15	80.49	40.70
T_2: NAA 200 ppm	6.17	25.50	261.00	11.60	5.60	85.50	40.40
T_3: NAA 300 ppm	6.80	26.40	274.00	10.70	5.40	71.40	40.00
T_4: Ethrel 150 ppm	5.50	24.40	240.00	14.70	6.30	76.55	49.00
T_5: Ethrel 300 ppm	5.00	23.13	226.60	16.18	7.16	68.51	47.50
T_6: Ethrel 450 ppm	4.53	22.01	215.00	17.25	7.60	65.60	46.55
T_7: CCC 100 ppm	4.50	19.92	225.56	18.50	8.20	78.23	56.05
T_8: CCC 200 ppm	4.40	18.98	214.80	18.78	8.32	73.81	54.00
T_9: CCC 300 ppm	4.36	18.05	203.26	20.97	9.30	70.18	52.00
T_{10}: Thiourea 250 ppm	5.79	25.82	243.05	13.39	6.14	75.80	45.79
T_{11}: Thiourea 500 ppm	5.47	23.35	258.10	11.58	5.25	70.77	41.20
T_{12}: Thiourea 750 ppm	5.00	22.15	258.90	11.70	5.41	70.68	41.17
SEm±	0.35	1.23	10.95	0.75	0.34	4.33	1.98
CD at 0.05%	1.01	3.58	31.95	2.19	0.98	12.63	5.79

Table 2. Impact of plant growth regulators and thiourea on physio-chemical quality and yield parameters of bottle gourd [Lagenaria siceraria (Mol.) cv. Pusa Summer Prolific Long]

Treatment	Physio-chemical quality parameters			Yield parameters	
	TSS (^{0}B)	Crude protein contents	Ascorbic acid contents	Yield/vine (kg)	Yield/hectare (q)
T_0: Control	5.03	0.200	11.66	5.70	349.25
T_1: NAA 100 ppm	5.07	0.210	12.10	6.15	378.19
T_2: NAA 200 ppm	5.17	0.217	12.40	6.40	396.76
T_3: NAA 300 ppm	5.21	0.220	12.50	6.95	472.55
T_4: Ethrel 150 ppm	5.16	0.216	12.46	7.02	499.15
T_5: Ethrel 300 ppm	5.26	0.222	12.75	7.45	533.86
T_6: Ethrel 450 ppm	5.31	0.226	12.90	7.55	549.84
T_7: CCC 100 ppm	5.14	0.215	12.35	6.14	426.35
T_8: CCC 200 ppm	5.23	0.221	12.65	6.40	447.96
T_9: CCC 300 ppm	5.28	0.224	12.82	6.70	473.42
T_{10}: Thiourea 250 ppm	5.15	0.211	12.25	6.08	377.26
T_{11}: Thiourea 500 ppm	5.18	0.212	12.21	6.30	393.71
T_{12}: Thiourea 750 ppm	5.20	0.219	12.49	6.65	416.32
SEm±	0.24	0.011	0.42	0.32	18.97
CD at 0.05%	NS	0.033	NS	0.94	55.36

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SHORT COMMUNICATION

STUDY OF ECONOMICS ON MAIZE (*ZEA MAYS* L.) INFLUENCED BY WEED MANAGEMENT

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Abstract: A field investigation was conducted at BAU experimental Farm, Ranchi during *kharif* season 2015 on sandy clay loam soil. The experiment was laid out in a RBD with 13 treatments: pretilachlor 1.0 kgha^{-1} PE (T_1),atrazine 1.0 kgha^{-1} PE (T_2),pendimethalin 1.0 kgha^{-1} PE (T_3), metribuzin 0.35 kgha^{-1} PE (T_4),pretilachlor 0.5 + metribuzin 0.175 kgha^{-1} PE (T_5), atrazine 0.5 + pendimethalin 0.5 kgha^{-1} PE (T_6), pretilachlor 1.0 kgha^{-1} at 15 DAS (T_7), metribuzin 0.35 kgha^{-1} at 15 DAS (T_8), atrazine 1.0 kgha^{-1} at 15 DAS (T_9), green manuring by *Sesbania* @ 80 kgha^{-1} *fb* 2,4-D 0.625 kgha^{-1} at 30 DAS (T_{10}), two mechanical weeding at 20 and 40 DAS (T_{11}), two hand weeding at 20 and 40 DAS (T_{12}),and weedy Check (T_{13}), replicated thrice. Results revealed that gross return (70591Rs.ha^{-1}), net return (44623Rs.ha^{-1}) and B: C ratio (1.72) were observed maximum due to application of same treatment (atrazine 0.5 + pendimethalin 0.5 Rs.ha^{-1}PE). The cost of treatment (atrazine 0.5 + pendimethalin 0.5 kg ha^{-1}PE) is much lower (1768 Rs.ha^{-1}) against mechanical weedings (3749 Rs.ha^{-1}) and hand weedings (9372 Kg.ha^{-1}).

Keywords: Maize, Weed management, Economics, Investigation

INTRODUCTION

Maize (Zea mays L.) is one of the most versatile crops having wider adaptability under diverse soil and climatic conditions. Globally, maize is known as the "Queen of Cereals" because it has the highest genetic yield potential amongst the cereals owing to its better dry matter accumulation efficiency in a unit area and time particularly up to 30° North and 30° South latitude. It is cultivated in an area of about 150 M ha in 160 countries in diverse soil types, climate, and management practices with wider plant biodiversity that contributes about 36% towards the global food grain production (Anonymous, 2013a). It is the third most important crop of India after rice and wheat that occupies an area of about 8.67 M ha with an average productivity of about 2.57 t/ha compared to the world average productivity of about 4.94 t ha^{-1} (Anonymous, 2014). It is grown on an area of 1.86 m ha with an average productivity of 1.45 t ha^{-1} in Jharkhand (Anonymous, 2013b). Yield losses caused due to different weed categories in maize were quantified as 77.4% by grassy, 44.2% by broad-leaf and 38.4% by sedges, indicating the need for weed management for realizing optimal crop yields.Manual weeding is exhaustive, lengthy, economically not feasible and laborious. Availability of man power for manual weeding at critical period of maize growth is difficult owing to pre-occupied farm work in other crops like rice, pulses etc. For controlling weeds in maize crop, pre-emergence or early post-emergence application of atrazine depending upon the soil type has been recommended. Application of pendimethalin also has been recommended under maize + legume intercropping situations. These herbicides do not control hardy weed species like *Commelinabenghalensis*, *Ageratum conyzoides*and *Brachiariaramosa*as they appear late in the season. The infestation of these weeds is increasing day by day in the maize-growing areas of the state especially where the farmers are using atrazine year after year. So in order to widen the weed control spectrum, it is imperative to use combination of herbicides having different mode of action. After reviewing these facts a studies performed with the objective to find out the economics of maize as influenced by weed management practices.

MATERIAL AND METHOD

The experiment was carried out during *kharif*, 2015 at BAU experimental Farm, Ranchi on sandy clay loam soil. The experiment was laid out in a RBD with 13 treatments: pretilachlor 1.0 kgha^{-1} PE (T_1),atrazine 1.0 kgha^{-1} PE (T_2), pendimethalin 1.0 Kg.ha^{-1}PE (T_3), metribuzin 0.35 kgha^{-1} PE (T_4), pretilachlor 0.5 + metribuzin 0.175 kgha^{-1} PE (T_5),atrazine 0.5 + pendimethalin 0.5 kgha^{-1} PE (T_6), pretilachlor 1.0 kgha^{-1} at 15 DAS (T_7), metribuzin 0.35 kgha^{-1} at 15 DAS (T_8),atrazine 1.0 kgha^{-1} at 15 DAS (T_9), green manuring by *Sesbania* @ 80 kgha^{-1} *fb* 2,4-D 0.625 Kg.ha^{-1}at 30 DAS (T_{10}), two mechanical weeding at 20 and 40 DAS (T_{11}), two hand weeding at 20 and 40 DAS (T_{12}),and weedy Check (T_{13}), replicated thrice.Maize *var.*Suwan was sown (on 30-06-2015) with spacing of 70 x 20 cm, seed rate 20 kgha^{-1} and RDF 120:60:40 kgha^{-1}.Gross return and cost of cultivation was calculated for each treatment, using current purchase price of inputs and the selling price of outputs prevailing in local market. Net profit was calculated as gross income subtracted

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by cost of cultivation. Benefit cost ratio was computed as the ratio of net return and cost of cultivation.

RESULT AND DISCUSSION

Economics

Economics of maize production as influenced by weed management practices are presented in Table 1. It was observed that percent increase (highest) in gross return, net return and B: C ratio was to the tune of 44.30, 71.10 and 70.93%, respectively due to application of atrazine 0.5 + pendimethalin 0.5 kg/ha PE (T_6) in comparison to weedy check (T_{13}).

Cost of treatment with application of atrazine 0.5 + pendimethalin 0.5 kgha^{-1} PE (T_6) was lower (1768 Rs ha^{-1}) compared to normal practice of hand weedings at 20 and 40 DAS (9372 Rs ha^{-1}) and resulted cost of cultivation 25969 and 33572 Rs ha^{-1}, respectively. Application of atrazine 0.5 + pendimethalin 0.5 kgha^{-1} PE (T_6) recorded maximum gross return (70,591 Rs ha^{-1}) which was *at par* with two hand weedings at 20 and 40 DAS (T_{12}), two mechanical weedings at 20 and 40 DAS (T_{11}), atrazine 1.0 kgha^{-1} PE (T_2) and pretilachlor 0.5 + metribuzin 0.175 kgha^{-1} PE (T_5). Weedy check recorded significantly lowest gross return (39,318 Rs ha^{-1}) and also it recorded significantly highest net return (44,623Rs ha^{-1}) as compared to rest of the other treatments. Weedy check recorded significantly lowest net return (15,117 Rs ha^{-1}) and significantly highest benefit: cost ratio (1.72) was observed with application of atrazine 0.5 + pendimethalin 0.5 kg ha^{-1} PE (T_6) as compared to rest of the other treatments. Weedy check recorded significantly lowest benefit: cost ratio (0.63).Similar results were also reported by Walia *et al.* (2007), Shantveerayya *et al*. (2013), Shankar *et al*. (2015) and Barla*et al.* (2016).

Table 1. Effect of weed management practices on economics (Rs ha^{-1}) and B: C ratio in maize (*var.* Suwan) during *kharif*, 2015.

	Treatments	Cost of weed management practice (Rs ha^{-1})	*Cost of cultivation (Rs ha^{-1})	Gross return (Rs ha^{-1})	Net return (Rs ha^{-1})	B:C ratio
T_1	Pretilachlor 1.0 kgha^{-1} PE	1695	25895	50152	24256	0.94
T_2	Atrazine 1.0 kgha^{-1} PE	1295	25495	59399	33903	1.33
T_3	Pendimethalin 1.0 kgha^{-1} PE	2241	26442	52822	26380	1.00
T_4	Metribuzin 0. kgha^{-1} PE	1950	26150	50481	24330	0.93
T_5	Pretilachlor 0.5 + metribuzin 0.175 kgha^{-1} PE	1637	25838	58935	33097	1.28
T_6	Atrazine 0.5 + pendimethalin 0.5 kgha^{-1} PE	1768	25969	70591	44623	1.72
T_7	Pretilachlor 1.0 kgha^{-1} at 15 DAS	1695	25895	49160	23264	0.90
T_8	Metribuzin 0.35 kgha^{-1} at 15 DAS	1950	26150	53814	27663	1.06
T_9	Atrazine 1.0 kgha^{-1} at 15 DAS	2375	26575	50941	24366	0.92
T_{10}	Green manuringfb 2, 4-D 0.625 kg/ha at 30 DAS	4573	28774	46832	18059	0.63
T_{11}	Mechanical weeding at 20 and 40 DAS	3749	27949	61696	33747	1.21
T_{12}	Hand weeding at 20 and 40 DAS	9372	33572	63944	30372	0.91
T_{13}	Weedy check	0	24201	39318	15117	0.63
SEm ±		-	-	5298.00	2273.67	0.08
CD (P=0.05)		-	-	15462.19	6635.69	0.22
CV%		-	-	16.85	14.20	12.60

*Includes cost of weed management practice.

CONCLUSION

Based on the results of present investigation, it can be concluded that application of atrazine 0.5 + pendimethalin 0.5 kgha^{-1} PE can be practiced as an economical weed management practice in maize (*Zea mays* L.) for higher productivity.

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