RESEARCH ARTICLE

# DIVA-GIS ANALYSIS ON GEOGRAPHIC DIVERGENCE OF COWPEA GERMPLASM FOR RESISTANCE TO PULSE BEETLE, *CALLOSOBRUCHUS CHINENSIS* L.

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**Abstract:** Twenty four cowpea genotypes collected from different regions of Andhra Pradesh, Odisha, Tamil Nadu and Telangana States were evaluated for their reaction against pulse beetle, *Callosobruchus chinensis.* Seed traits *viz*., seed length, seed width, test seed weight and insect biological parameters *viz*., number of eggs laid/20 seeds, number of adults emerged/20 seeds, percent adult emergence, mean developmental period, insect growth index and percent seed weight loss were used to assess the spatial distribution and diversity in the reaction of cowpea germplasm against *C. chinensis* using DIVA-GIS applications. Traits *viz*., percent adult emergence, growth index and number of eggs/ 20 seeds exhibited high variability as evidenced by high co-efficient of variation (CV) of 27.3%, 26.45% and 23.9% respectively. Moderately low CV values were observed for mean developmental period (0.83%) and number of adults emerged/20 seeds (4.74%). The study revealed that Adilabad district of Telangana and Srikakulam district of Andhra Pradesh were found highly diverse for all the traits including seed traits with high index values (1.433 – 2.000 for majority of the traits). The present findings would enable to identify the sources of resistance in cowpea germplasm geographically and spatially through grid maps.

**Keywords:** Cowpea, *Callosobruchus chinensis,* Cowpea resistance, DIVA-GIS, Geographical distribution

## INTRODUCTION

Pulse beetle, *Callosobruchus chinensis* (L.) is a key pest of stored cowpea (*Vigna unguiculata* (L) Walp.) in India and abroad. It has been reported that cowpea grains, which are not stored with either chemical or non-chemical methods, are often completely destroyed by bruchids to the extent of 95-100 per cent both quantitatively and qualitatively making them unfit for planting, marketing and human consumption (Mahendran and Mohan, 2002; Ali *et al*., 2004). Pest control measures in stored grains including legumes generally rely on the use of synthetic insecticides and fumigants, which resulted in insecticide residues on the treated crops, thus make them unfit for human consumption. In order to reduce both over dependence on chemicals for control and seed loss due to the bruchid attack, the search for host plant resistance in leguminous crops has increasingly become the option of choice in recent years. Several studies have been conducted to assess the resistance of cowpea genotypes to bruchids (Singh *et al.*, 1985; Azeez and Pitan, 2014; Kpoviessi *et al.,* 2020; Tripathi *et al.,* 2020). So far, only few cowpea accessions were found to offer resistance to bruchids (Singh *et al*., 1985). However, efforts must continue to identify new sources of insect resistant traits in vast cowpea germplasm so as to incorporate them in cowpea crop improvement programmes to evolve resistant or tolerant varieties against bruchids. In this context, the present investigation was carried out to evaluate cowpea genotypes for their reaction to *C. chinensis* and to map the diversity of resistance in the screened cowpea genotypes using DIVA-GIS to elucidate spatial and geographic patterns in the distribution of resistance to *C. chinensis*. The results emerged out of the present study would enable to identify the sources of resistance in cowpea germplasm geographically and spatially through grid maps.

## MATERIALS AND METHODS

In the present study, 24 cowpea genotypes (land races) collected from different locations of Andhra Pradesh, Odisha, Tamil Nadu and Telangana States and maintained in Medium Term Module (MTM) facility of ICAR-National Bureau of Plant Genetic Resources, Regional Station, Hyderabad were evaluated for their resistance to *C. chinensis* following standard no-choice method using a completely randomized design under laboratory conditions. Culture of *C. chinensis* was maintained on the cowpea seeds (local variety) at 28±1°C and 65±5.0 per cent RH in a Biological Oxygen Demand incubator. For this test, 20 healthy and dried seeds genotype of each were weighed and placed in small transparent plastic jars having perforated lids to ensure aeration. Two pairs of (male and female) of freshly emerged adults from the stock cultures were released in each jar for oviposition. Each jar was considered as one replication for each accession and was replicated for four times. After three days of allowance for oviposition, the insects were removed and numbers of eggs laid by the females on seeds of different accessions were counted to determine the level of oviposition. Later, all the jars were kept for observation under the same conditions as mentioned above until the emergence of adults. Adult emergence was initiated in about 25 days. Various

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observations *viz*., total number of eggs laid, adult emergence, development period, number of emergence holes and weight loss were recorded. Observations on adult emergence were recorded at regular interval of 24 h and continued until zero emergences were recorded so as to determine development period. Based on the observations, various parameters *viz*., percent adult emergence (PAE), mean development period (MDP) and growth index were calculated as suggested by Howe (1971) and Jackai and Singh (1988). The experimental seeds were weighed (X1) before releasing the insects for egg laying and were re-weighed after the emergence of adults (X2). The loss in seed weight as a result of feeding activity of the bruchid was calculated (X1-X2) and expressed in percentage.

Physical parameters of cowpea genotypes *viz*., seed coat colour and texture were recorded using different descriptors of IBPGR. Seed length and width and roundedness were measured using a Vernier Callipers and expressed in millimetres (mm). Seed weight was recorded by weighing 100 uniformly sized seeds using an analytical balance and expressed in grams.

DIVA-GIS version 7.5.0 (www.DIVA-GIS.org) was used to assess the spatial distribution and diversity in cowpea germplasm for their reaction against bruchids. India shape file was used for generating maps. Maps on the spatial analysis diversity were generated from a point-to-grid analysis using the simple method. All analyses were based on the geo-referenced points and additional attributes of point data recorded for cowpea accessions *viz*., number of eggs laid/20 seeds, number of adults emerged/20 seeds, percent adult emergence (PAE), mean developmental period-days (MDP), growth index (GI) and percent seed weight loss (PSWL).

Statistical analysis of data was carried out by a single factor ANOVA. The data on number of adults emerged and mean developmental period (MDP) were square root transformed and data on percent seed weight loss (PSWL) and percent adult emergence (PAE) were angular transformed before analysis. Analyses of variance were carried out using DSAASTAT, version, 1.1 statistical package (Onofri, 2007) available at http://www.unipg.it/~onofri/DSAASTAT/DSAASTAT.htm. The Least Significant Difference (LSD) values at $P = 0.05$ were used to determine the significance of treatment mean differences.

## RESULTS AND DISCUSSION

Cowpea's adaptability to different types of soil and intercropping systems, its resistance to drought, and its ability to improve soil fertility and prevent erosion makes it an important economic crop in India and other countries. The cowpea germplasm (24 genotypes) used in the present study were collected from four Indian States *viz*., Andhra Pradesh, Odisha, Tamil Nadu and Telangana covering 11 districts in the South East Coastal zone (Table 1). The range of variability in seed traits and other experimental traits recorded on cowpea germplasm were seed length (5.3-8.0 mm), seed width (4.6-6.0 mm), test seed weight (7.0-14.2 g), number of eggs laid/20seeds (50.25-146.25), number of adults emerged/20seeds (17-20.75), percent adult emergence (13.22-41.94), mean developmental period (27.66-28.7 days), growth index (0.47-1.4) and percent seed weight loss (28.38-46.63), with mean values of 6.6, 5.19, 10.57, 86.24, 18.73, 24.76, 28.28, 0.87 and 39.11 respectively (Table 2). Majority of the traits studied on cowpeas' reaction against bruchid exhibited variability evident by high co efficient of variation (CV) as observed in number of eggs laid per twenty seeds (23.9%), percent adult emergence (27.3%) and growth index (26.45%). Moderately low CV values were observed for mean developmental period (0.83%) and number of adults emerged per 20 seeds (4.74%).

Grid maps were generated using the DIVA-GIS for the bruchid reaction traits on cowpea recorded in the laboratory, which depicted the distribution pattern *viz*., number of eggs laid/20 seeds (Fig.1), number of adults emerged/20 seeds (Fig.2), percent adult emergence (Fig.3), mean developmental period (Fig.4), growth index (Fig.5) and percent seed weight loss (Fig.6). The grid maps revealed that Adilabad district of Telangana and Srikakulam district of Andhra Pradesh were found highly diverse for all the traits including seed traits with high index values (*e.g.* 1.433 – 2.000 for majority of the traits and 0.9 - 2.0 for number of adults emerged per 20 seeds and seed colour). Interestingly, the analysis of diversity for cowpea germplasm seed colour indicated that diverse lines occur in Chittoor (A.P.) and Medak (Telangana) in addition to Srikakulam (A.P) and Adilabad (Telangana) (Fig.7). Similarly, the grid map generated for seed test weight (Fig.8) revealed diverse lines are available in Adilabad, Srikakulam, Chittoor, Medak and Visakhapatnam districts. Further exploration can be undertaken in Adilabad (Telangana) and Srikakulam (Andhra Pradesh) districts for identifying good sources of resistance in cowpea for *C. chinensis*.

Divergence on the basis of the traits studied showed that the main cowpea phylogenetic tree was divided into two main sub-clusters (A & B) out of which 12 samples of cowpea were grouped together in cluster A and B respectively (Fig. 9). The genotypes of the cluster A showed high genetic distance than that of the cluster B. A careful observation of the cluster A revealed that it was divided further into two major sub-clusters (Sub-cluster 1A and 2A.) Cluster 1A had 11 cowpea accessions (IC628780, IC582880, IC519603, IC343899, IC582853, IC519815, IC519699, IC282059, IC282032, IC282058 & IC519762) while the sub-cluster 2A consists of only one accession (IC257844) which were closely related genetically. The main cluster B had been further divided into two sub-clusters viz., 1B and 2B respectively. The sub-cluster 1B consists of 7 cowpea genotypes (IC436804, IC261240, IC436845, IC398985, IC519750, IC51972018, IC399004) while the sub-cluster 2B with 5 genotypes (IC519766, IC436734, IC436897, IC519570, IC436683) respectively (Fig. 9).

The results of the study, based on biological parameters *viz.*, AE, GI and PSWL on the cowpea germplasm revealed that none of cowpea accession was found be to immune or resistant to *C. chinensis*, but most of them, were either susceptible or highly susceptible. It is stated further that the cowpea accession IC 257844 was categorized as moderately resistant and seven were classified as moderately susceptible. Biological parameters like number of eggs laid, adult emergence, mean developmental period (MDP), growth index (GI) and PSWL are widely used globally by researchers to identify the reaction of various legume crops to bruchid infestation (Ileke *et al*., 2013; Tripathi *et al*., 2015; Gopalaswamy *et al*., 2019; Mohamed *et al*., 2019; Kpoviessi *et al.,* 2020; Tripathi *et al*., 2020). The use of GIS technology has had considerable impact on pest management. Though its potential is not fully comprehended in plant protection arena, few attempts were made to utilize the tool in pest mapping and modelling. Ganeshaiah *et al*., (2003) successfully used DIVA-GIS in predicting the potential distribution of sugarcane wooly aphid *Ceratovacuna lanigera* Zehntner in South India. The DIVA-GIS technique has been successfully adapted earlier by researchers in India for the diversity mapping and analysis of germplasm collections (Varaprasad *et al*., 2008; Sunil *et al*., 2009; Babu Abraham *et al.*, 2010; Sivaraj *et al*., 2010). The findings of the present study would be helpful to conduct exploration for identifying good sources of resistanct in cowpea for *C. chinensis*. Probably, this would be the first attempt to map the reaction of cowpea germplasm against bruchid in India using the geographical information system.

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**Table 1.** Source of cowpea landrace diversity used for the DIVA-GIS analysis

| **State** | **District** | **Latitude** | **Longitude** |
|---|---|---|---|
| Andhra Pradesh | Chittoor | 13.2172 | 79.1003 |
| | Chittoor | 13.2172 | 79.1003 |
| | Nellore | 14.4212 | 79.7345 |
| | Srikakulam | 18.273 | 84.0048 |
| | Srikakulum | 18.273 | 84.0048 |
| | Srikakulum | 18.273 | 84.0048 |
| | Srikakulum | 18.273 | 84.0048 |
| | Srikakulum | 18.273 | 84.0048 |
| | Srikakulum | 18.273 | 84.0048 |
| | Visakhapatnam | 17.8928 | 82.6904 |
| | Visakhapatnam | 17.6868 | 83.2185 |
| | Visakhapatnam | 17.6868 | 83.2158 |
| | Vizianagaram | 18.1067 | 83.3956 |
| Odisha | Gajapati | 19.1848 | 84.1608 |
| | Koraput | 18.8561 | 82.7347 |
| Tamil Nadu | Namakkal | 11.18 | 78.21 |
| Telangana | Adilabad | 19.253 | 78.977 |
| | Adilabad | 19.253 | 78.977 |

| | | | |
|---|---|---|---|
| | Adilabad | 19.6578 | 78.544 |
| | Adilabad | 19.253 | 78.977 |
| | Adilabad | 19.3675 | 78.7755 |
| | Medak | 17.8848 | 78.2132 |
| | Medak | 17.8848 | 78.2132 |
| | Nalgonda | 17.0665 | 79.2884 |

**Table 2.** Descriptive statistics on seed traits and reaction against bruchids

| Trait | Minimum | Maximum | Range | Mean | Standard Deviation | Coefficient of Variaion (CV%) |
|---|---|---|---|---|---|---|
| Seed length (mm) | 5.3 | 8 | 2.7 | 6.60 | 0.78 | 11.78 |
| Seed width (mm) | 4.6 | 6 | 1.4 | 5.19 | 0.38 | 7.23 |
| Seed weight (g) | 7 | 14.2 | 7.2 | 10.57 | 1.87 | 17.66 |
| Eggs laid/20seeds | 50.25 | 146.25 | 96 | 86.24 | 20.61 | 23.90 |
| Adults emerged/20seeds | 17 | 20.75 | 3.75 | 18.73 | 0.89 | 4.74 |
| Percent adult emergence (PAE) | 13.22 | 41.94 | 28.72 | 24.76 | 6.76 | 27.30 |
| Mean developmental period (MDP)(days) | 27.66 | 28.7 | 1.04 | 28.28 | 0.23 | 0.83 |
| Growth index (GI) | 0.47 | 1.4 | 0.93 | 0.87 | 0.23 | 26.45 |
| Percent seed weight loss (PSWL) | 28.38 | 46.63 | 18.25 | 39.11 | 4.64 | 11.87 |

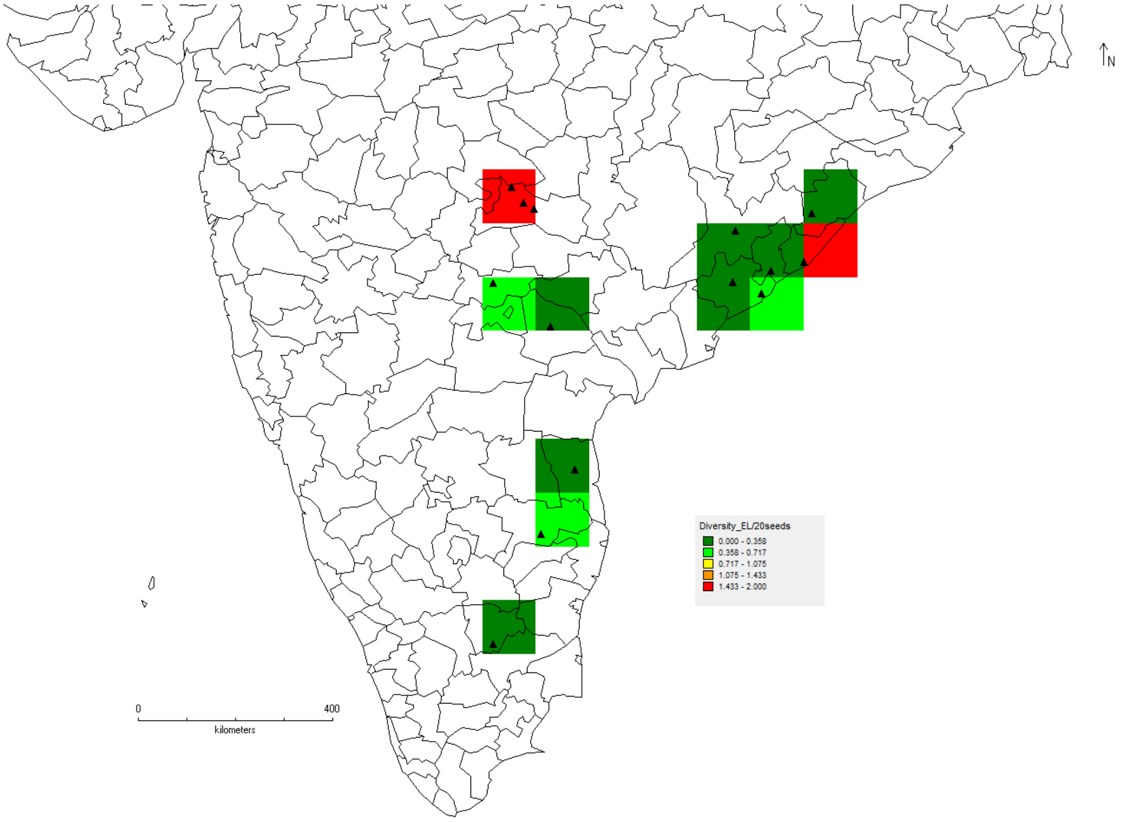



**Fig. 1.** Analysis of diversity for number of eggs laid per 20 seeds in cowpea

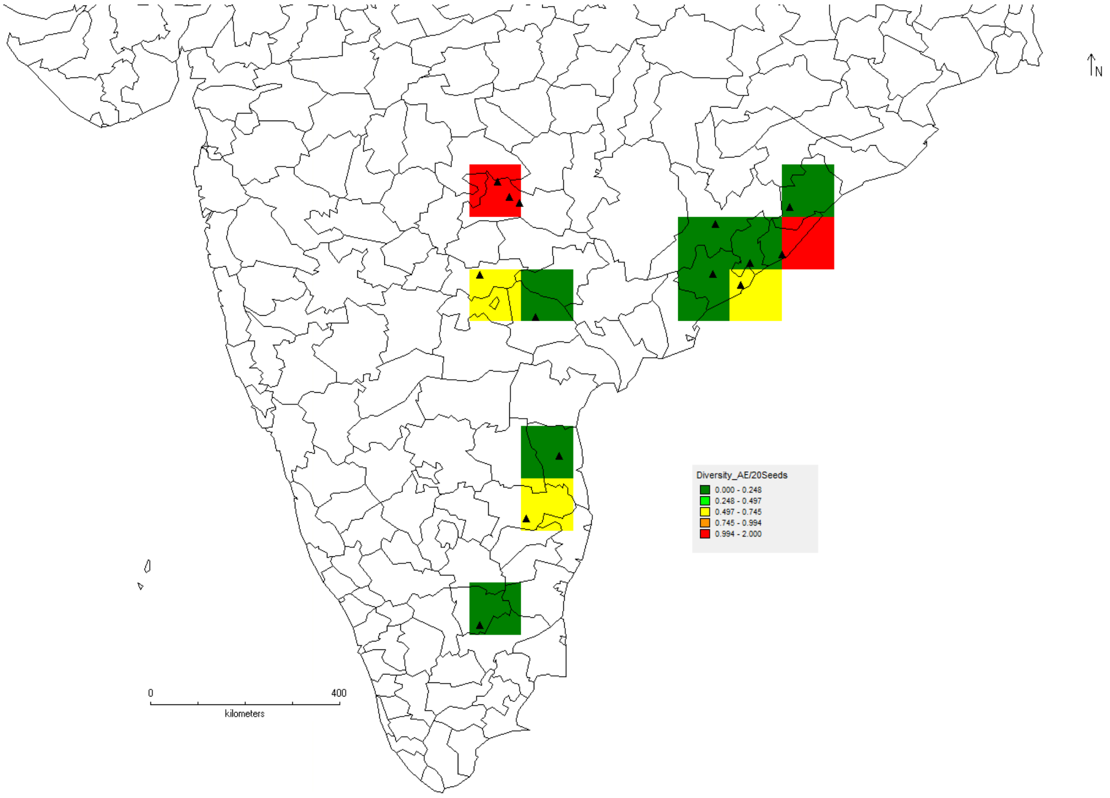



**Fig. 2.** Analysis of diversity for number of adults emerged per 20 seeds in cowpea

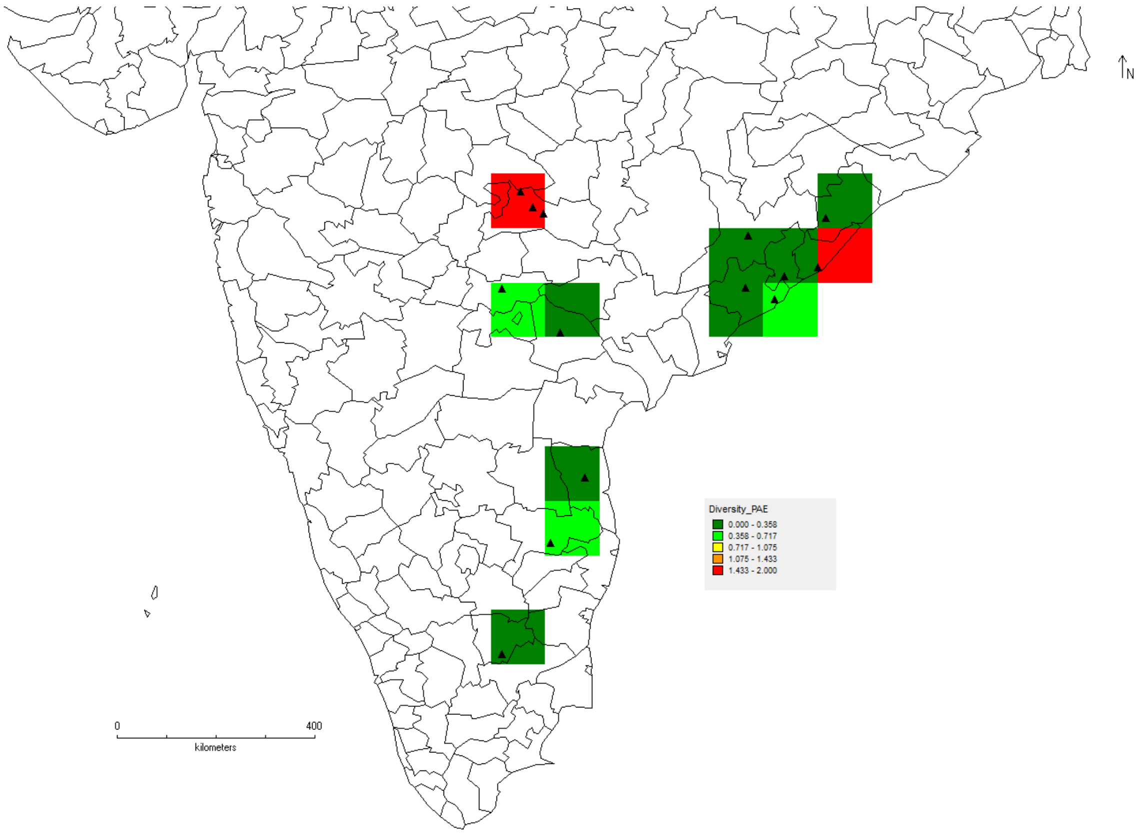



**Fig. 3.** Analysis of diversity for percent adult emergence (PAE) in cowpea

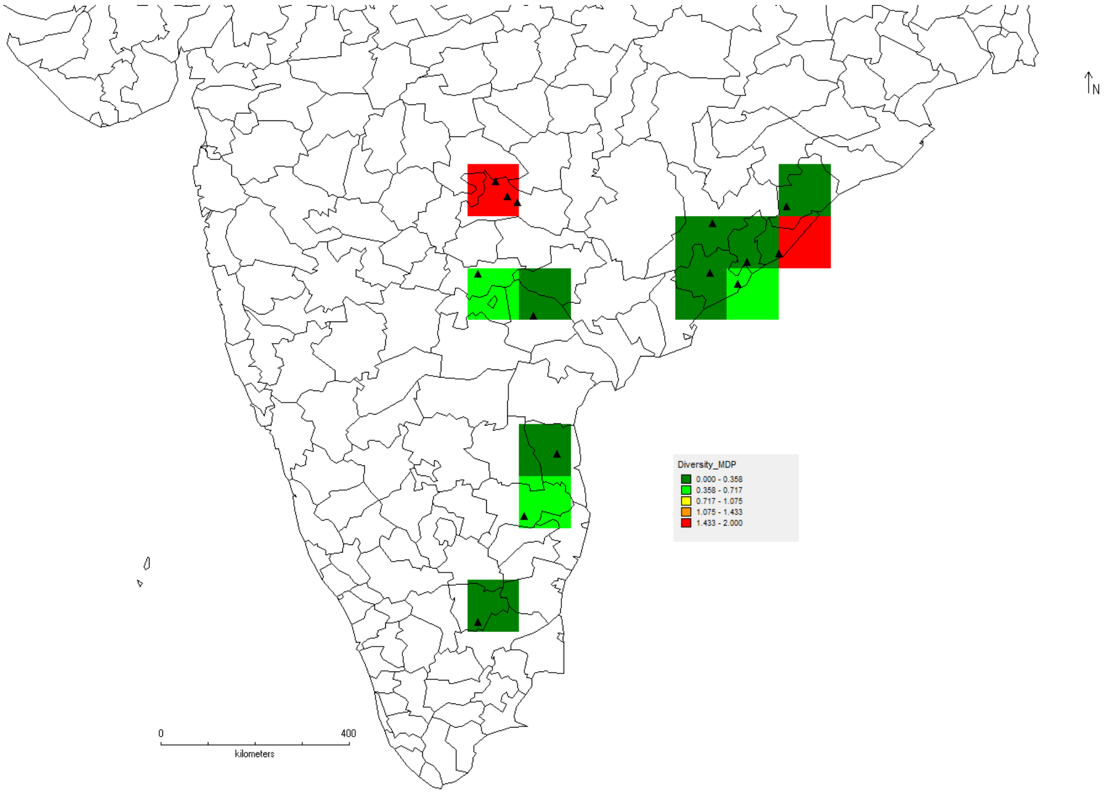



**Fig. 4.** Analysis of diversity for mean developmental period (MDP)

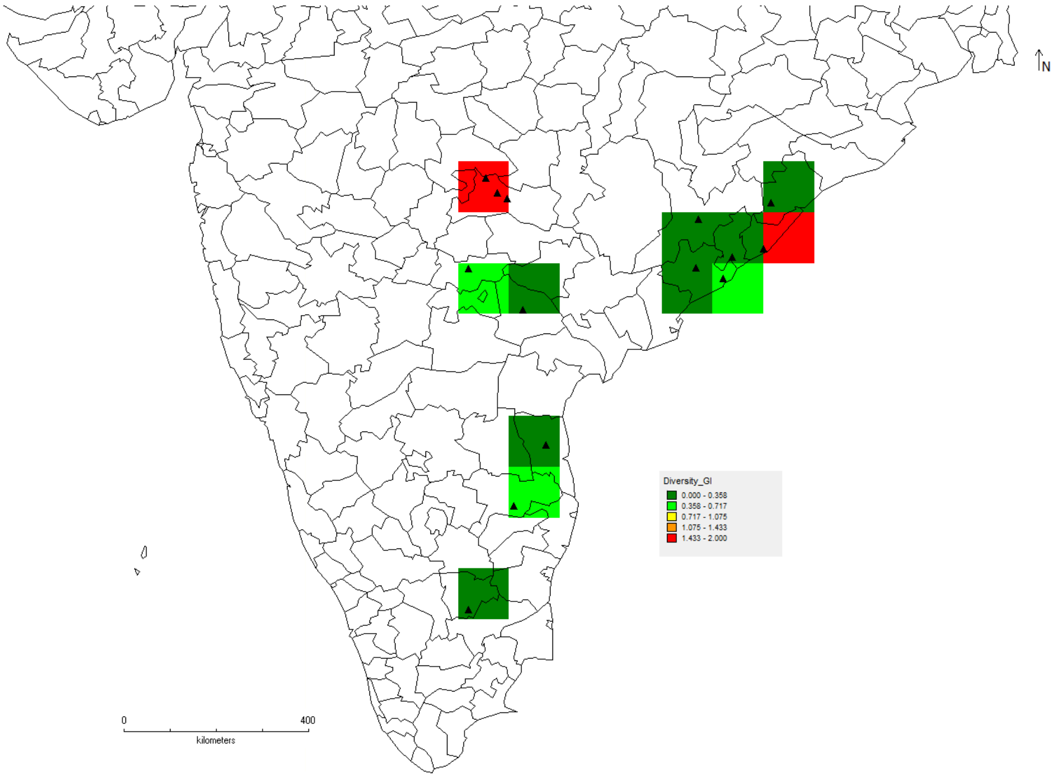



**Fig. 5.** Analysis of diversity for growth index

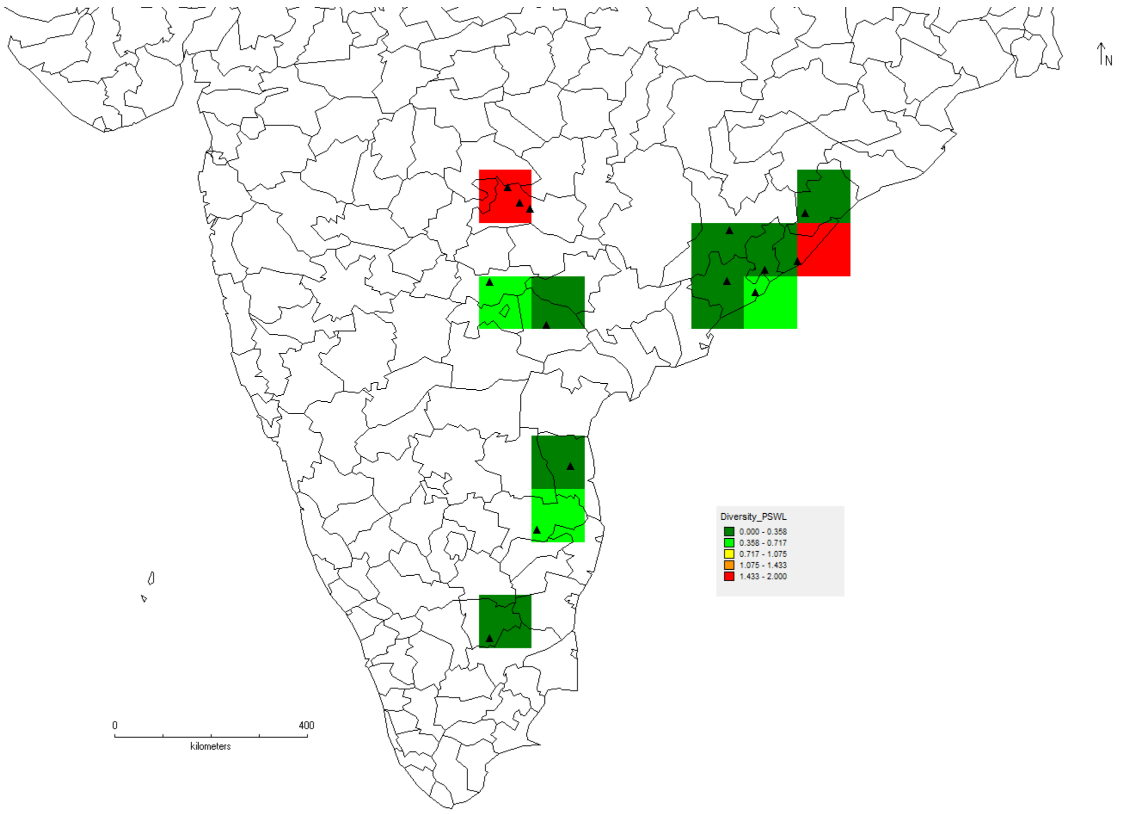



**Fig. 6.** Analysis of diversity for percent seed weight loss in cowpea

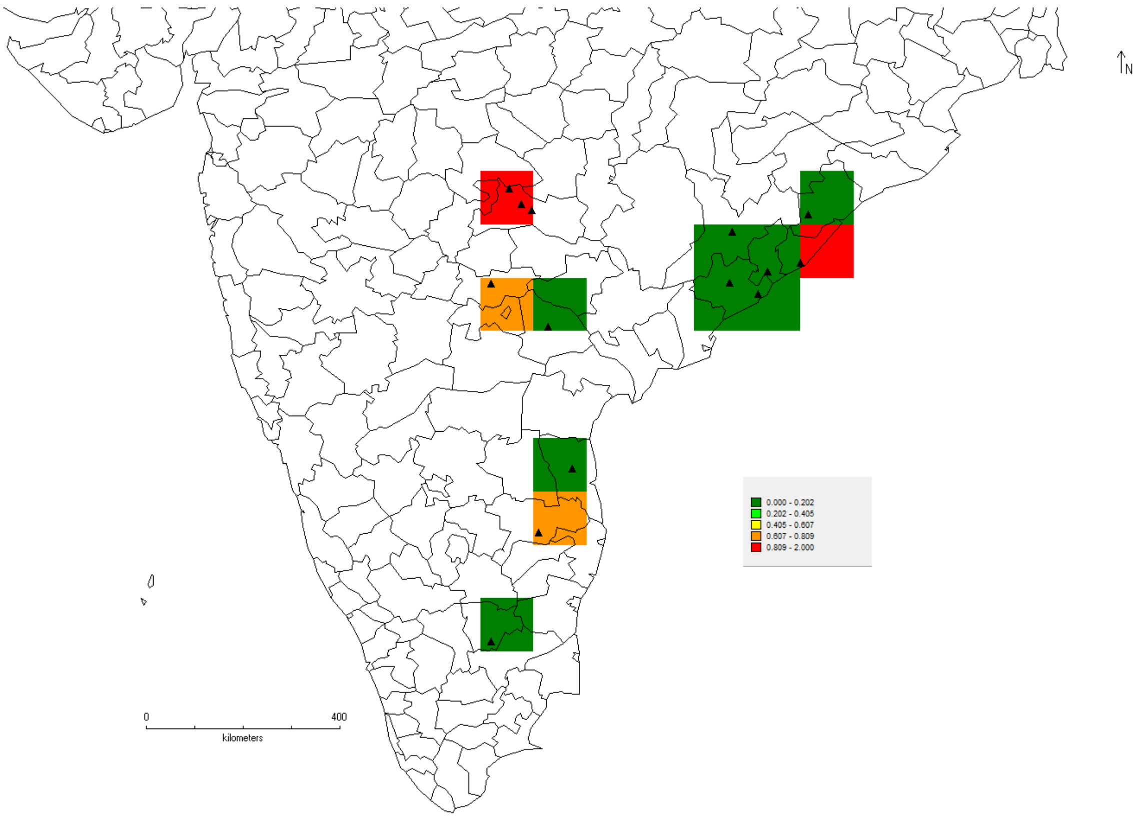



**Fig. 7.** Analysis of diversity for seed colour in cowpea

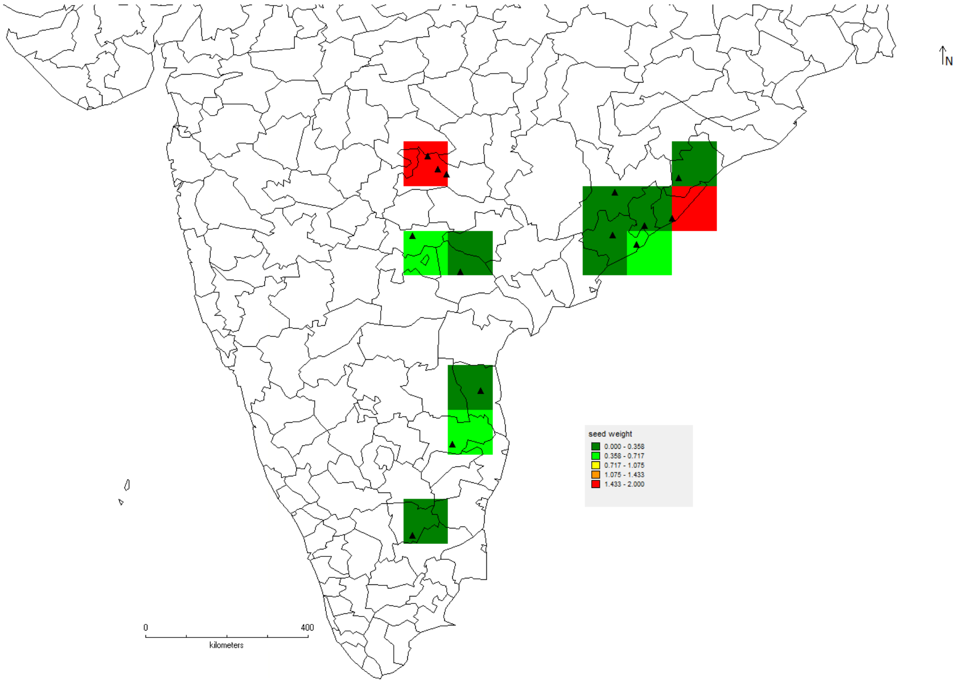
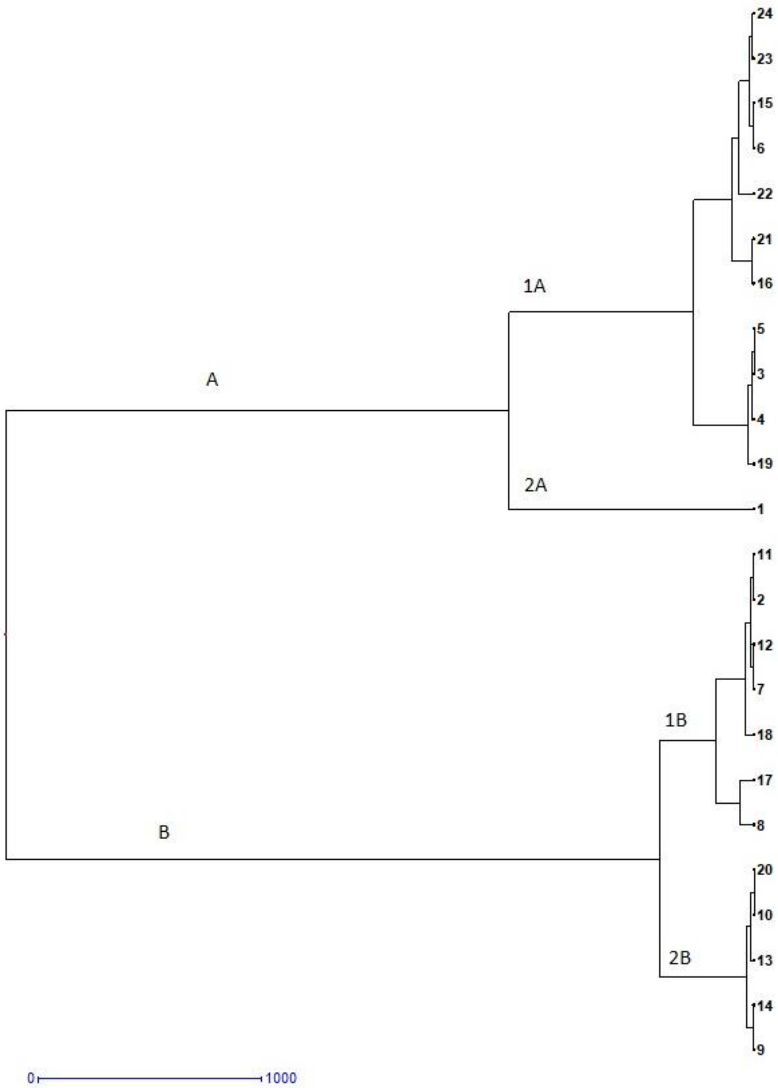



**Fig. 8.** Analysis of diversity for test seed weight in cowpea



1. IC257844, 2. IC261240, 3. IC282032, 4. IC282058, 5. IC282059, 6. IC343899, 7. IC398985, 8. IC399004, 9. IC436683
10. IC436734, 11. IC436804, 12. IC436845, 13. IC436897, 14. IC519570, 15. IC519603, 16. IC519699, 17. IC519720
18. IC519750, 19. IC519762, 20. IC519766, 21. IC519815, 22. IC582853, 23. IC582880, 24. IC628780

**Fig. 9.** Ward's Minimum variance dendrogram generated for Cowpea

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