RESEARCH ARTICLE

COMPARATIVE STUDIES OF DIFFERENT GENOTYPES OF *TRITICUMAESTIVUM* FOR CALLUS INDUCTION AND REGENERATION USING DIFFERENT GENERATIVE EXPLANTS, MEDIA AND PHYSICAL AND CHEMICAL MUTAGENS

Anju Sharma, Sudhir Sharma* and Ramesh Kumar

**Assistant Scientist, Department of Genetics & Plant Breeding, CCSHAU,RRS, Uchani, Karnal*
CCS Haryana Agricultural University, Hisar – 125004

Received-08.10.2020, Revised-29.10.2020

Abstract: This paper compared the behavior of diverse set of wheat genotypes in their tissue culture response.Significant differences were detected in plant callusing,culture efficiency and regeneration capacity when immature embryo (non treated or EMS treated) and matureembryo(NS,ES,30KR(ES) and 35KR(ES) of six wheat cultivars were compared. In immature embryo callus induction was significantly higher on 2, 4-D (Raj3765 98.4%) supplemented medium than 2, 4, 5-T (Raj3765 89.2%) in case of non treated while in EMS treated one it was higher on 2, 4, 5-T (Raj3765 88.3%) containing medium. Non treated and EMS treated immature embryos showed significant differences and better was in non treated explants. Regeneration was highest in PBW 343 (82.7%) when non treated callus were transferred to regeneration media. Similar type of response was observed with mature embryos for callus induction as it was highest in NS(UP2338 100%) among all ES system (non treated (UP2338 87.90%), 30KR (WH542 87.3%) and 35KR gamma irradiated (Raj3765 88.6%).Regeneration was best in 30KR(ES) mature embryo derived callus among all (PBW343 92.5%).PBW343 was the best genotype regarding regeneration in mature embryo(NS PBW343 91.4%),ES non treated (DI9 83.3%).Mature embryo was superior explant than immature embryo for callusing and regeneration.

Keywords: Callus induction, Media, Mutagens, Regeneration, *Triticum aestivum*

INTRODUCTION

Wheat (*Triticum aestivum* L.) is one of the most important staple food as well as feed crops in many parts of the world. Though India is the world's second largest producer of wheat .Hence, there is an urgent need to produce wheat cultivars that are adaptable to diverse biotic and abiotic challenges. Wheat improvement requires introduction of novel as well as introduce variation through tissue culture. Plant regeneration from cultured cells and tissues is required for the successful application of biotechnology in crop improvement (*Tomar et al.,* 2011 and *Chawla et al.,* 2015). Immature tissues like immature embryos are most preferred explants for somaclonalvariation (*Ozgen et al.,* 1998 and *Sharma et al.,* 2003) because of their high regeneration capacity. However useof immature embryos demands extra labour and expense for maintaining the donor plants. Additionally, their most suitable stage for culture also limits their use. Mature embryos or a tissue derived from them has been used as an effective alternative to immature embryos because of their year round availability and easy isolation. Furthermore, the physiological state of mature embryos shows minimal variability (*Varshney et al.,* 1999 and *Parmar et al.,* 2012). However, some new techniques such as endosperm supported callus induction method have been successfully used for callus induction from mature embryo cultures (*Jia et al.,* 2008 and *Sharma et al.,* 2017). Callus induction and regeneration in wheat have been reported to be cultivar specific .In the present study we aimed at improving the callus development and regeneration with response to gamma irradiation and EMS treatment in six wheat cultivars for further use in development of somaclones.

Abbrevations

1. E.M.S. – Ethyl methane sulphonate
2. NS – Non endosperm supported
3. ES- Endosperm supported
4. KR- Kilo Rads

MATERIALS AND METHODS

Immature embryos were obtained after 16-21 days of anthesis from field grown plants of non treated and EMS (0.5%, 4Hr.) treated seeds. Mature embryos obtained from treated seeds with 30KR and 35KR gamma irradiation and non treated seeds after washing with Tween-80 and rinsed with fresh water 6-7 times and soaked overnight. Immature embryos were sterilized with 0.1% $HgCl_2$ for 5-6 min followed by 4-5 washing with sterilized distilled water. Mature embryos (seeds) sterilized with $HgCl_2$ for 8 minutes followed by washing with sterilized distilled water (5 times) were used.

Establishment of callus culture from different explants

Immature embryos

The excised immature embryos were cultured on MS medium with 5mg/l 2,4-D + 0.2mg/l NAA(M1) and MS medium with 5mg/l 2,4,5-T + 0.2mg/l NAA(M2) and subculturing was done(8times) on MS medium with 3mg/l 2,4-D(M3) after every 4 weeks

*Corresponding Author

interval. 40-60 embryos/genotype/medium were cultured with 3 embryos in one tube and scutellum of embryos facing away from the surface of growth medium.

Mature embryos

Mature embryos from gamma ray treated (30KR and 35KR) and non treated seeds were aseptically moved (not set free) slightly and with furrow downwards as well as completely remove embryos were placed on MS medium with 7mg/l 2, 4-D + 0.2mg/l NAA (M4).After 10 days the developed calli were subcultured on MS medium with 5mg/l 2, 4-D (M5). About 36 mature embryos (3 embryos per test tube) were considered as the unit of replication as each experiment had three replications.

Regeneration

The regeneration calli of immature embryo were transferred to MS medium with 1mg/l BAP+ 0.2mg/l IAA(M6) while mature embryos calli on MS medium with 0.1mg/l NAA(M7).All the cultures for regeneration were kept under 16Hr.photoperiod.The cultures were examined periodically.

The data were analysed by using completely randomized design (CRD) of angular transformed values.

Table 1. Effect of callus initiation medium and EMS treatment on callus induction from immature embryos and regeneration percentage in different wheat cultivars

Sr. No.	Genotypes	Callus Induction				Regeneration M_1 (Non treatedcalli)	No. of shoots per callus
		Non-treated		EMS treated			
		M_1	M_2	M_1	M_2		
1	DI9	87.06 (69.42)	83.0 (66.19)	64.06 (53.21)	87.06 (68.96)	76.8 (61.34)	8-12
2	DP 2338	71.7 (57.95)	65.8 (54.25)	80.3 (63.91)	71.6 (57.93)	76.9 (61.44)	4-5
3	Raj 3765	98.4 (85.87)	89.2 (71.61)	79.61 (64.23)	88.3 (70.15)	76.7 (61.25)	5-6
4	WH 147	96.4 (83.67)	85.6 (68.41)	86.03 (68.16)	87.16 (69.68)	66.5 (54.48)	6-8
5	PBW 343	85.7 (68.15)	86.2 (68.29)	77.13 (61.44)	87.7 (70.08)	82.7 (66.14)	8-10
6	WH 542	84.6 (67.19)	83.9 (66.35)	81.9 (64.96)	83.3 (65.95)	79.3 (63.21)	4-5
	Total Mean	**87.31 (72.04)**	**82.28 (65.85)**	**78.18 (62.65)**	**84.19 (67.13)**	**76.4 (61.31)**	
CD	**A**	**3.78**					
	B	**4.63**		**NS**			
	A×B	**9.27**					

Figures in parentheses are angular transformed values.

Table 2. Comparison of callus induction frequency between NS and ES mature embryos and among ES non treated, ES with 30 kR and ES with 35 kR treated mature embryo of different wheat genotypes

Genotypes	Non treated mature embryo (NS)	Non treated Mature embryo (ES)	30kR treated mature embryo (ES)	35 kR treated mature embryo (ES)
DI9	72.00 (58.74)	66.4 (54.70)	74.3 (59.60)	76.8 (61.10)
DP 2338	100.00 (90.00)	87.9 (69.83)	84.9 (66.89)	70.3 (57.7)
Raj 3765	70.3 (57.10)	86.6 (68.85)	69.2 (56.32)	88.6 (70.97)
WH 147	88.8 (70.49)	62.9 (52.52)	69.0 (56.34)	79.5 (62.92)
PBW 343	91.7 (73.29)	70.3 (57.07)	73.4 (59.08)	62.9 (52.50)
WH 542	56.6 (483.93)	59.2 (50.35)	87.3 (69.64)	70.3 (57.02)
Total Mean	**79.90 (66.43)**	**72.22 (58.89)**	**76.35 (61.31)**	**74.73 (60.27)**
CD	**A**	**2.87**		
	B	**3.51**		
	A×B	**7.02**		

Figures in parentheses are angular transformed values.

Table 3. Shoot regeneration response of mature embryo calli derived from different systems (NS,ES) and gamma irradiation treatments (30 kR, 35 kR) in different wheat genotypes

Genotypes	Non treated mature embryo (NS)	Non treated Mature embryo (ES)	30kR treated mature embryo (ES)	35 kR treated mature embryo (ES)
DI9	78.03 (62.58)	83.3 (65.99)	83.3 (66.15)	75.0 (60.51)
DP 2338	78.03 (62.06)	60.2 (50.92)	90.0 (75.0)	86.6 (68.85)
Raj 3765	86.50 (68.54)	76.16 (60.86)	90.5 (76.20)	72.3 (58.54)
WH 147	85.13 (67.46)	52.3 (46.49)	87.5 (69.68)	75.0 (60.02)
PBW 343	91.4 (76.11)	79.6 (63.78)	92.5 (76.95)	70.6 (57.19)
WH 542	80.5 (63.92)	71.5 (57.78)	86.6 (68.81)	82.2 (65.36)
Total Mean	**83.27** (66.78)	**70.51** (54.64)	**88.4** (72.13)	**76.95** (61.74)
CD	**A**	**4.55**		
	B	**NS**		
	A×B	**11.13**		

NS = Non endosperm supported system; ES = Endosperm supported system.
Figures in parentheses are angular transformed values.

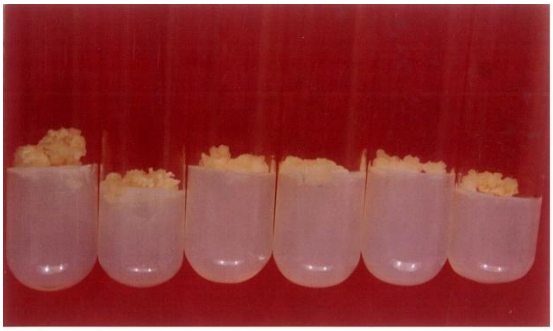

Fig. 1

Fig. 2

Fig. 3

Fig. 4

RESULTS

Callus was raised from immature embryos after 4 days of inoculation(Fig. 1).The immature embryos (non treated and EMS treated) of all the genotypes began to swell on both the media tested(M1 and M2).Non treated immature embryos formed white, nodular and embryogenic callus with 71.7 to 98.4 % callus induction(Table 1). Raj3765 showed best callusing (98.4%) followed by WH147 (96.4%) on M1 medium while on M2 medium it was 65.8 to 89.2% and highest callusing was found in Raj3765(89.2%) followed by PBW343(86.2%).Significant differences between M1(87.3%) and M2 (82.2%) medium were observed in non treated immature embryo.EMS treated immature embryos formed yellowish white and friable callus. Callus induction percentage was 64 to

86% for M1 medium and highest callus induction was reported in WH147 (86.0%) followed by WH 542(81.9%).While on M2 medium Raj 3765 exhibited highest callusing (88.3%) followed by PBW343 (87.7%).Callus induction was significantly higher (84.1%) on M2 medium than M1 medium(78.1%) in EMS treated immature embryos and non treated immature embryos showed significantly higher callusing(87.3%) than EMS treated one (78.1%) when cultured on M1 medium. Significant genotypic differences were also reported for EMS treated and non treated as well as M1 and M2 medium.M1 derived calli were transferred to regeneration medium. Shoot regeneration took place within 6-7 days after inoculation. Regeneration was 76.4% and range was 66.5 to 82.7%.Highest regeneration was reported in PBW343 (82.7%) followed by WH542(79.3%).Number of shoots per callus was ranged 5-12 and highest was in DI9 (Fig 2).Regenerated plants were transferred to potted soil and grown to maturity with 76% survival and collect the seeds.

Mature embryo

Callus formation took place within 2 days after inoculation from endosperm supported mature embryos (ES) of non treated and gamma irradiated (30KR and 35KR seeds in all genotypes) but 6 days in NS.Callus was whitish and watery. The callus induction range was 59.2 to 87.9% in non treated endosperm supported (ES) mature embryos. Highest callus induction was obtained in UP2338 (87.9%) followed by Raj3765 (86.6%) (Table 2).Mature embryo of 30KR seeds in ES system exhibited callus induction of 76.3% and range was 69.0 to 87.3%.Highest callusing was observed in WH542(87.3%) followed by UP2338(84.9%) while in 35KR seeds derived mature embryos callus induction was 74.7% and callus induction range was 62.9 to 88.6%.However,highest callus induction was reported in Raj3765(88.6%) followed by WH147(79.5%).After 10 days of inoculation ,size of the callus reached or surpassed that of the seeds and they became suitable for subculture(Fig 3).Nevertheless, callus size varied according to the genotype and Raj3765 was best in this respect followed by DI9 in all cases(non treated 30KR and 35KR treated mature embryo in endosperm supported system).The percentage of non endosperm supported mature embryos (NS) for callus induction was 56.6 to 100 % and highest was obtained from UP2338 (100%) followed by PBW343 (91.7%). Callus was compact, white, nodular and embryogenic. Callus induction was significantly higher in NS than ES but in ES callus initiation was very early(after 2 days) in comparison to NS system(after 6 days).Among ES system mature embryos of 30KR seeds exhibited significantly higher callus induction (76.3%) than non treated ES system(72.2%).Non significant differences was observed for callus induction between 30KR mature embryos (76.3%) and 35KR mature embryos(74.7%).Significant genotypic differences was also observed for callus induction between NS and non treated ES system. Among ES system also (non treated and 30KR and 35KR treated seeds mature embryos) genotypes exhibited significant differences.

Regeneration

The regeneration took place within 6 days when calli of all types were transferred to regeneration medium(Fig 4).The average of regeneration percentage (83.2%) in non treated NS system derived calli which was significantly higher than non treated ES system derived calli(70.5%) as well as 35KR ES system derived calli(76.9%) while regeneration percentage was significantly highest in 30KR ES system(88.4%) among the all non treated ES (70.5%),35KR ES (76.9%) and NS system derived calli(83.2%) genotypes were not exhibited significant differences with the treatments for regeneration potential. Regeneration percentage ranged from 78.0 to 91.4% in NS system derived calli(Table 3) and highest was observed in PBW343(91.4%) followed by Raj3765(86.5%) while in non treated ES system calli it was 52.3 to 83.3 % and highest was in DI9 (83.3%) followed by PBW343(79.6%).Regeneration range was 83.3 to 92.5% in 30KR ES system and highest was observed in PBW343(92.5%) followed by Raj3765(90.5%).However in 35KR ES system it was 70.6 to 86.6 % and highest was obtained in UP2338(86.6%) followed by WH542(82.2%) there were observed significant differences also in genotype x treatment. Healthy and well developed plants were transferred to potted soil and survival was 80% .Seeds was collected from these plants to make appropriate observation for somaclonal variation.

DISCUSSION

The choice of explants is the most important criteria for successful cell and tissue culture protocols. Mature embryo is reported to be one of the good sources of primary explants for in vitro regeneration of wheat (*Sharma et al.,* 2017).According to *Ozgen et al.* (1998) mature embryos have great advantage to be used as explants for wheat tissue culture as it has high potentiality for callus induction and regeneration. In the present study gamma irradiation exhibited positive effect for callusing as well as regeneration. Mutagenesis generates genetic variation whatever expressed in the phenotype or in DNA sequences .The variation in genes is a means of adding value to genetic resources (*Sharma et al.,*2019). In this study we reported callus initiation within 4 days from immature embryos while it took only 2 days in mature embryos. Callus was whitish and watery from gamma irradiated seed but it was compact, white, nodular and embryogenic from non treated non endosperm supported mature embryo.

Regeneration took place within 7 days from both immature and mature embryo. *He* (2008) reported the callus induction upto 97.6% from mature embryo of NS in Barley while we reported upto 100% callus induction from mature embryo of NS.

CONCLUSION

Callusing response of mature embryo was significantly higher in non treated NS than ES while early callus initiation and growth was in ES system .Efficiency of 30KR gamma irradiation was better than 35KR for callusing and regeneration both. There was significant difference for callus induction among tissue source x genotypes x EMS treatment in immature embryo.

REFERENCES

Chawla, A., Cheena, P. and Chaudhary, S. (2015). Effect of cytokinin preconditioning on in vitro multiple shoot regeneration of Lentil cultivar. *J Plant Dev.Sci*.Vol.7(8): 613-618.

Ozgen, M., Turet, M., Altinok, S. and Sancak, C. (1998). Efficient Callus induction and regeneration mature embryo culture of winter wheat(*Triticum aestivum* L.) genotypes. *Plant cell Reports*.18: 331-335

Parmar, S.S., Sainger, M., Chaudhary, D. and Jaiwal, P.K. (2012). Plant regeneration from mature embryo of commercial Indian bread wheat (*Triticum aestivum* L.) cultivars. *Physiol.Mol.Bio.Plants* 18(2):177-183

Sharma, A., Sharma, S. and Kaushik, A. (2017). A new method to increase callus induction and plant regeneration from mature embryo of wheat. *J.Pharmacogasy.Phytochem*. 6:2658-2661

Sharma, A., Uppal, S., Kumar, S. and Sharma, S. (2003). Response of wheat immature embryo cultures to ethyl methane sulphonatetreatment.*Natnl.J.Pl.Improv*. 5(2):77-79

Sharma, S., Kumar, P. and Yadav, M. (2019). Development of drought tolerant wheat through mutagenic and plant tissue culture tools. *International Research J. of Bio.Sci*. Vol.1: No.(1):4-12

He, T. and Jia, J.F. (2008). High frequency plant regeneration from mature embryo explants of highland barley (*HordeumVulgare*L.var.nudum HKF) under endosperm supported culture.*Plant Cell Tiss.Organ Cult*. 95:251-254.

Tomar, R., Dahiya, B.S., Malik, A. and Kumar, N. (2011). Plant regeneration through callus tissue of Okra(*Abelmoschusesculentus*.L.Moench).*J Plant Dev.Sci*. Vol.3 (1-2): 193-198.

Varshney, A., Jain, S. and Kothari, S.L. (1999). Plant regeneration from mature embryos of 20 cultivars of wheat (*Triticumaestivum* L. and *Triticum durum* desf.) *Cereal Res.Commu*.27 (1-2): 163-170.