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Research Paper

Mature Seed based Plant Regeneration system in Wheat (*Triticum aestivum* L)

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Abstract

Mature seed based plant regeneration is the most desirable one. Since it is convient to collect independent of growing season and developmental stages and consistent in physiological status. These advantages make mature seed based explants most easily handled material for plant transformation. In this paper the information based on mature seed explants regeneration is collated and data on screening bread wheat and durum wheat is presented.

Key Words: callus, wheat, mature seeds, mature embryos, germinating seedlings and coleoptilar nodes

Introduction

Unlike the model plant, wheat has remained to be difficult in the transgenic study, mainly due to the lack of explants with high regeneration efficiency. So far, the regeneration of wheat plantlets has been successfully achieved *in vitro* from its immature embryos, inflorescences, anthers, mature embryos (Chugh and Khurana 2003, Patnaik et al. 2006, Ding et al. 2009 and Parmar et al. 2012) and microspores among which the regeneration efficiency for different genotypes is 0-80% from the immature embryo culture (Bohorova et al 1995, Fennell et al. 1996), 0-50% from the anther culture (EI- Hennawy et al 2011) and 0-20% from the mature embryo culture

(Chugh and Khurana 2003; Patnaik et al. 2006; Ding et al. 2009 and Parmar et al. 2012). Therefore, the immature embryos of wheat have been mostly used as receptor explants in transformation (Vasil *et al* 1992; Parmar et al. 2012). However, collection of the immature embryos is strictly limited by environmental conditions and growth periods. In addition, the physiological status of these explants suitable for tissue culture and transformation is greatly affected by the growth conditions such as temperature, light, nutrition, water, and diseases. Moreover, using immature embryos is so time-consuming that it is hard to meet the needs of the transgenic study of wheat. In comparison, the mature embryos are convenient to collect, independent of growing seasons and development stages and consistent in physiological status. These advantages made the mature embryos the most easily handling materials for plant transformation. Therefore, it is very important to establish an efficient regeneration system for the wheat mature embryos to promote the research in the molecular breeding and functional genomics. Several researchers have reported

callus induction and plant regeneration from mature seed based explant in wheat (Table 1). Two wheat varieties namely PBW222 and PBW215 were used in present study with the objective to check their callous induction and regeneration potential from mature seed based explants.

Table 1. Studies on Mature Seed based Plant Regeneration System in Wheat

Explant Type	Medium	Reference
Mature seed/Mature embryo explants	C = LS+2, 4-D R = LS + IAA+BA	Mackinnon *et al* 1987
Leaf base culture	C = MS + 2, 4-D R = Basal MS medium	Rajyalakshmi *et al*. 1991
Mature embryo (12 common winter wheat varieties)	C = MS + 2, 4-D (8 mg/l) R = Basal MS medium	Ozgen *et al* 1998
Mature seed	C = LS + 2, 4-D R = Basal MS medium	Malik *et al* 2003
Mature embryo (CPAN 1676 & PDW215)	C = MS + 2, 4-D R = MS+BAP+IAA	Patnaik & Khurana 2003
Shoot tips	C = T-medium+2,4-D B5 + 2,4-D R = MS+BAP+NAA	Sharma *et al* 2003
Basal segment of 2-4 day old seedlings	C = MS + 2, 4-D R = MS, 2 , 4-D, BAP, NAA	Wang *et al* 2004
Mature embryo (Several varieties)	C = MS + 2, 4-D R = Basal MS medium	Ahmet *et al* 2007
Leaf based cultures	C = MS + 2, 4-D (Pulse) R = Basal MS medium	Mahalakshmi *et al* 2007
Mature embryo	C = MS + 2, 4-D R = MS+BAP+$AgNO_3$	Yu *et al* 2008
Mature embryo	C = MS + 2, 4-D R = MS+IAA+Kin.+2iP	Afzal *et al* 2010
Mature embryo	C = MS + 2, 4-D, Picloram, Dicamba R = Basal MS or with TDZ, Zeatin	Parmar et al. 2012

Material and Methods

Two wheat varieties viz. PBW 222 and PDW 215 were used in the present study. Four mature seed based explants viz. mature seeds as such, embryos excised from mature seeds, germinating seedlings and coleoptilar nodes were cultured on different callusing medium(Table2).

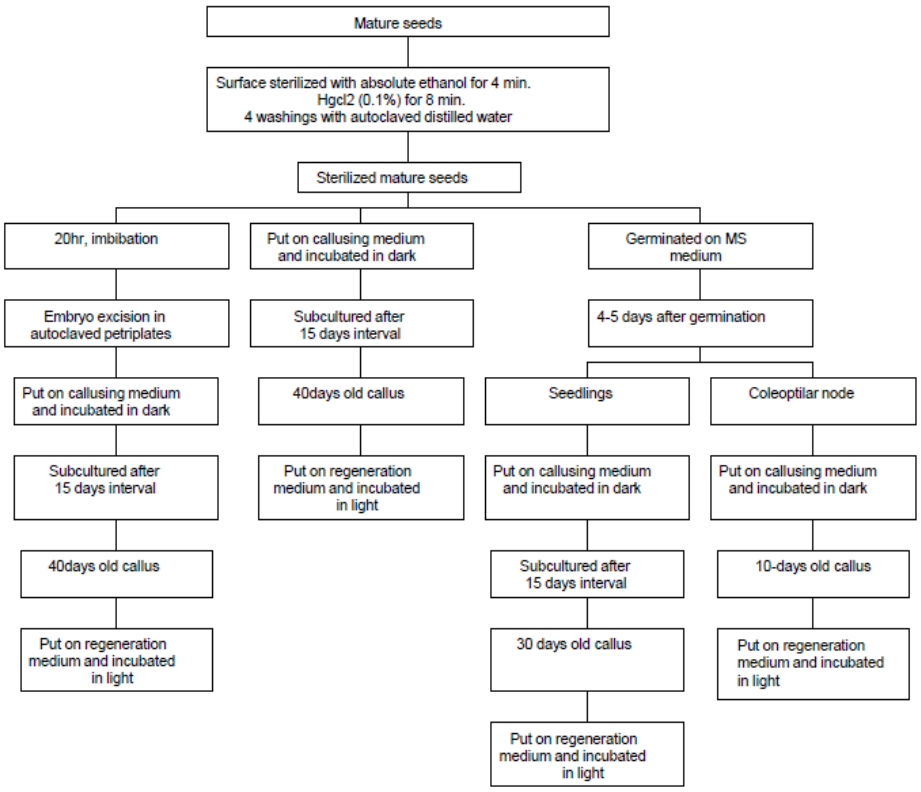
Table (2) Composition of Various Media used

Media	Composition
Callusing Media	
CM2	MS+Asparagine+Thiamie+ Proline +2,4-D (150mg/l) (40mg/l) (10mg/l) (2.5mg/l)
CM2-5	MS+Asparagine+Thiamie+2,4-D (150mg/l) (40mg/l) (5mg/l)

CM2-10	MS+Asparagine+Thiamie+2,4-D (150mg/l) (40mg/l) (10mg/l)
CM3	MS+Asparagine+Glutamine+Proline+2,4-D (150mg/l) (25mg/l) (10mg/l) (2.5mg/l)
Regeneration Media	
RM1	MS+Asparagine+Thiamie+IAA + BAP (150mg/l) (40mg/l) (0.2mg/l) (1mg/l)
RM2	MS+Asparagine+Thiamie+Proline + IAA + BAP (150mg/l) (40mg/l) (10mg/l)(0.2mg/l) (1mg/l)
RM3	MS+Asparagine+Glutaimine+Proline +IAA + BAP (150mg/l) (25mg/l) (10mg/l) (0.2mg/l) (1mg/l)

Surface sterilization and preparation of explants: As shown in flow chart (Fig.1) the healthy seeds were selected. Seeds were surface sterilized in sterile air provided by laminar air flow using autoclaved flasks/test tubes. Sterilization was done by first dipping the seeds in absolute alcohol for four minutes followed by 0.1 % $HgCl_2$ treatment for eight minutes with constant shaking. The sterilized seeds were cultured on callusing media with their grooved surface on one side and were kept in total dark. For excision of embryos the sterilized seeds were soaked in autoclaved distilled water for 6-20 hours to soften the seed coat. These embryos were cultured on callusing media as such or by dividing it into two parts with alongitudinal cut. For leaves from germinating seedlings the sterilized seeds were placed on basal MS medium for germination. After 4-5 days of germination, the seedlings were cut into 1cm long pieces and cultured on callusing medium amd incubated in dark. Three days after germination, the coleoptilar nodes (the small amorphous tissue from which shoots and roots arise) were separated from the sterilized seeds with the help of forceps and scalpel blade and were cultured on callusing medium and incubated in dark.

Fig 1. CULTURING PROCEDURES FOR MATURE SEED BASED EXPLANTS



Results

Mature embryos

The seeds were soaked into autoclaved distilled water before excision of embryos for 6-20 hours. The embryos could be excised after 6h imbibitions period, which was the minimum time required for softening of seed coat and removal of embedded embryos from endosperm. But the imbibition for 20h was found to be suitable for better regeneration response from the embryos. Imbibition for longer time was not suitable for better excision, because of excessive softening of seeds.

Two wheat varieties one of durum wheat i.e. PDW 215 and one of bread wheat i.e. PBW 222 were used to study the callusing and regeneration response of mature embryos. The scutellum of the mature embryos did not form callus. The callus in mature embryos originate from embryonic axis. Table (3) shows the callusing response of mature embryos on different media - CM2 (2.5mg/l), CM2-5 (2,4-D 5mg/l), CM2-10 (2,4-D 10mg/l) and CM3. Higher concentrations of 2,4-D (5-10 mg/l) did not enhance callus induction. However these concentrations reduced the precocious germination. In PDW 215 the percent callus induction on CM2, CM2-5, CM2-10 and CM3 was 85.3%, 75.8%, 55.6% and 79.3% respectively. Whereas, in PBW 222 the percent callus induction on CM2, CM2-5, CM2-10 and CM3 was 80%, 71.3%, 78.4% and 72.7% respectively. The calli were maintained for forty five days on callusing medium before their transfer to regeneration medium.

Table (3) Percent Callusing and Shoot Regeneration from Mature Embryos of Two Tissue Culture Responsive Genotypes of Wheat on Four Callusing Media.

Genotypes	CM2	CM2-5	CM2-10	CM3	Average
PDW215	85.3 (28.6)	75.8 (18.2)	55.6 (0.0)	73.9 (41.2)	72.20 (20.7)
PBW222	80.0 (31.8)	8103 (0.0)	78.4 (0.0)	72.7 (2.9)	78.57 (16.1)

Figures in parantheses are the regeneration response

For shoot regeneration, the calli were transferred to regeneration media RM2 and RM3 and kept in florescent light. Table (3) shows the shoot regeneration response. In PDW 215 the calli induced on CM2, CM2-5 and CM2-10 exhibited 28.6%, 18.2% and zero regeneration, respectively, upon transfer to RM2 shoot regeneration medium. Similarly in PBW 222 calli induced on CM2, CM2-5, CM2-10 exhibited 31.8% and zero regeneration , respectively, upon transfer to RM2 medium. Calli of PDW 215 and PBW 222 obtained on CM3 medium gave 41.2% and 42.9% regeneration, respectively on RM3 regeneration medium.

Mature Seeds

Seeds of two varieties PDW 215 and PBW 222 were cultured on four callusing media. Table (4) shows the callusing response of these two varieties on four media i.e. CM2 (2.5mg/l), CM2-5 (2,4-D 5mg/l), CM2-10 (2,4-D 10mg/l) CM3. PDW 215 gave 57.17%, 50.9%, 41.8% and 49.2% callusing while PBW 222 gave 50%, 53.1%, 46.2% and 52.3% callusing on CM2, CM2-5, CM2-10 and CM3 media, respectively.

Table (4) Percent Callusing and Shoot Regeneration from Mature Seeds of Two Tissue Culture Responsive Genotypes of Wheat on Four Callusing Media.

Genotypes	CM2	CM2-5	CM2-10	CM3	Average
PDW215	57.1 (34.8)	50.9 (14.8)	41.8 (0.0)	49.2 (33.3)	49.1 (19.4)
PBW222	50.0 (33.3)	53.1 (11.5)	46.2 (0.0)	52.3 (33.3)	50.4 (21.4)

Figures in parantheses are the regeneration response

Table (4) shows the regeneration response of the calli obtained on media CM2, CM2-5 and CM3. Calli of PDW 215 obtained on CM2 exhibited 34.8%, while calli of PBW 222 exhibited 33.3% shoot regeneration on RM2. Calli of PDW 215 induced on CM2-5 exhibited 14.8% shoot regeneration while those of PBW 222 gave 11.5% shoot regeneration response on RM2. Calli of PDW 215 and PBW 222 induced on CM2-10 did not regenerate on RM2. Calli of PDW 215 and PBW 222 induced on CM3 callusing medium showed 33.3% shoot regeneration on RM3 regeneration medium.

Germinating Seedlings

Four best responsive genotypes of wheat i.e. PBW 343, PBW 222, PBW 226 and PBW 215 were used in this experiment. Leaves of the germinating seedlings were cut into pieces of 1cm length. These pieces were culture on callusing medium CM3. Table (4) shows the callusing response of these four genotypes on CM3 media. PDW 215 gave 87.8%, PBW 222 gave 88.2%, PBW 226 gave 78.1% and PBW 343 gave 80% callusing response.

Table (5) Percent Callusing and Shoot Regeneration from Leaves of Germinating Seedlings of Different Wheat Genotypes on CM3 and RM3 Media.

Genotypes	Callusing	Regeneration
PDW215	87.28	0.0
PBW222	88.20	0.0
PBW226	78.10	0.0
PBW343	80.00	0.0

Although there was profuse callusing from basal segments of seedling pieces, but no regeneration was observed from these calli.

Coleoptilar Node

Coleoptilar node of two tissue culture responsive genotypes PDW 215 and PBW 222 were put on calusing media. Table (6) shows callusing and regeneration response of these two genotypes. PDW 215 gave 94.7% callusing whereas, PBW 222 gave 88.9% callusing from coleoptilar node. Coleoptilar derived calli of PDW 215 showed 16.7% shoot regeneration on RM3 medium whereas the calli of PBW 222 did not regenerate into shoots.

Table (6) Percent Callusing and Shoot Regeneration from Coleptilar Node of Two Tissue Culture Responsive Wheat Genotypes.

Genotypes	Callusing	Regeneration
PDW215	94.7	16.7
PBW222	88.9	0.0

Disscussion

To obtain explant, which remains available round the year four mature seed based explants were tried i.e. mature seeds, mature embryos, leaves from germinating seedlings and coleoptilar node. In case of mature seed as explants and mature embryo as explants, two highly regenerative wheat varieties one of bread wheat, PBW 222 and one of durum wheat, PDW 215 were used. Two best responsive media for callusing, CM2 and CM3 were used. In case of CM2 callus induction medium, three different levels of 2,4-D i.e. 2.5mg/l, 5mg/l, and 10mg/l were used. Before dissection of mature embryos, seeds were soaked in water for 6-20 hours. 20 hours soaking of seed in water before isolation of mature embryos was found to be best. This was in accordance with some previous studies of Chylah *et al*. (1990). Mature embryos were placed on solid medium by keeping scutellar side down as discussed by Chylah et al. (1990). Mature seeds were cultured as such on solid medium. Table (3) shows the callusing response and plant regeneration response of two different varieties from mature embryos as explant. CM2 with 2.5mg/l 2,4-D gave best response for callus induction. But maximum regeneration was observed in calli induced on CM3 induction medium. Persual of data indicates that increase in the concentration of 2,4-D did not increase the callusing response but reduces the regeneration ability drastically. Calli produced on CM2 medium having 2,4-D (10mg/l) did not show any regeneration response. Table (4) shows the callus and plant regeneration response of two varieties from mature seed explants in two different media i.e. CM2 and CM3. Here also three levels of 2,4-D i.e. 2.5mg/l, 5mg/l and 10mg/l were tried in case of callus induction medium. Again CM2 medium gave best response for callus induction, whereas callus produced on CM2 as well as CM3 gave similar results for plant regeneration. Increased level of 2,4-D did not show any advantage in callusing, rather decreased the

regeneration potential. O'Hara and Street (1978) and Lazar *et al.* (1983) have reported plant regeneration from callus induced from mature embryos.

Leaves from germinating seedlings of four highly responsive genotypes were used for callusing and plant regeneration. Based on the previous experiments, only one medium i.e. CM3, which was found to be best responsive for regeneration used in this study. Approximately 1 cm long leaf pieces were cultured on callus induction medium CM3. In all the leaves, only basal one or two segments gave callusing response. Rajyalakshsmi *et al.* (1991) were also able to get calli from first one or two segments of leaves. So only two basal leaf segments were included for recording the observations. Table (5) shows callusing and regeneration response from leaves of germinating seedlings. Although very high callus induction response was there yet no regeneration was recorded. Contrary to this, Rajyalaksmi *et el* (1991) were able to regenerate plants from calli produced from leaf segments.

To study the callusing and regeneration response of coleoptilar node, it was dissected from two varieties, PDW 215 and PBW 222 as mentioned by Mchughen (1991). Coleoptilar nodes from these varieties were cultured on CM3 medium. Very high callusing response was observed for both the varieties i.e. 94.7% from PDW 215 and 88.9% from PBW 222. Calli produced from PDW 215 gave 16.7% shoot regeneration, where as no shoot regeneration was observed from PBW 222. Mchughen reported that regenerants from coleoptilar node derived callus reach anthesis about 60 days after initial culture of coleoptilar node.

REFERENCES

1. Afzal A, Rashid H, Khan M H, Chaudhry Z and Malik S A (2010) High frequency Regeneration system optimization for wheat cultivar Inqilab-91. *Pak J Bot* 42:1857-62.
2. Ahmet H and Adak S (2007) Callus induction and plant regeneration in some Iraqi common wheat varieties. *J Agrl Sci* 13:285-92.
3. Ascough, G.D., Bakos, F., Balazs, E., Barnabas, B., Van Staden, J., (2006). Screening South African wheat germplasm for androgenic competence. *S. Afr. J. Bot.* 72, 40–45.
4. Bohorova N E, Ginkel V M, Rajaram S and Hoisington D A (1995) Tissue culture response of CIMMYT elite bread wheat cultivars and evaluation of regenerated plants. *Cereal Research Communication* 23: 243-249.
5. Chugh A, Khurana P (2003) Regeneration via somatic embryogenesis from leaf basal segments and genetic transformation of bread and emmer wheat by particle bombardment. *Plant Cell Tiss Org Cult* 74:151–161
6. Chylah H , Hassaine R and Chylah A (1990)Improvement of somaic embryogenesis in wheat by segmentation of cultured embryos. In Bajaj, Y P S (ed.). *Biotechnology in Agriculture and forestry,* Vol. 13, wheat . Springer-Verlag Berlin Heildelberg, New York pp 88-97.
7. Ding L, Li S, Gao J, Wang Y, Yang G, He G (2009) Optimization of Agrobacterium-mediated transformation conditions in mature embryos of elite wheat. *Mol Biol Rep* 36:29–36.
8. EI- Hennawy M A, Abdalla A F, Shafey S A and Ashkar I M (2011) Production of doubled haploid wheat lines (*Triticum aestivum* L.) using anther culture technique. *Annals of Agricultural Science* 56(2), 63–72.
9. Fennell S, Bohorova N, Gimbel M and Hoisington (1996) Plant regeneration from immature embryos of forty eight elite CIMMYT bread wheats. *Theor. Appl. Genet.* 92:163-169.
10. Lazar M D, Collins G B and Vian W E (1983A F) Genetic and environmental effects on the growth and differentiation of wheat. *Somatic cell Culture* 74: 353-57.
11. Mackinnon C, Gunderson G and Nabros M W (1989) High efficiency plant regeneration by somatic embryogenesis from callus of mature embryo explants of bread wheat (*Triticum aestivum*) and grain sorghum. *In Vitro Cell Dev Biol Plant* 23:443-48.
12. Malik S I, Rashid H, Yasmin T and Minhas N M (2003) Effect of 2,4-D on callus induction from mature wheat seeds. *Int J Agric Biol* 6: 156-59.

13. Muchgen A (1983) Rapid regeneration of wheat *in vitro. Ann. Bot.* (London) 51:851-853.
14. O'Hara J F and Street H E (1978) Wheat callus culture: The initiation , growth and organogenesis of callus derived from various explants sources. *Ann Bot* 42: 1029-38.
15. Ogzen M, Turet M, Altmok S and Sancak C (1998) Efficient callus induction and plant regeneration from mature embryo culture of winter wheat genotypes. *Plant cell Rep* 18:331-35.
16. Parmar S S, Sainger M, Chaudhary D, Jaiwal P K. (2012) Plant regeneration from mature embryo of commercial Indian bread wheat (Triticum aestivum L.) cultivars. *Physiol Mol Biol Plants* 18(2):177–183
17. Patnaik D and Khurana P (2003) Genetic transformation of Indian bread and pasta wheat by particle bombardment of mature embryo-derived calli. *BMC Plant Biol* 3:5.
18. Patnaik D, Vishnudasan D, Khurana P (2006) Agrobacterium-mediated transformation of mature embryos of Triticum aestivum and Triticum durum. *Curr Sci* 91:307–317.
19. Rajyalakshmi K, Grover A, Maheswari N, Tyagi A K and Maheswari S C (1991)High frequency regeneration of plantlets from leaf bases via somatic embryogenesis and comparison of polypeptide profiles from morphogenic and non morphogenic calli in wheat (*Triticum aestivum*). *Physiol Planatarum* 82:617-23.
20. Sharma A, Uppal S, Sharma S and Sharma S K (2003) An alternative source for regenerable embryogenic callus induction from shoot tips of wheat (*Triticum aestivum* L.) *Ind J Genet Plant Breed* 63:209-11.
21. Vasil V, Castilo A M, Fromm M E and Vasil I K (1992) Herbicide resistant fertile transgenic wheat plants obtained by microprojectile bombardment of regenerable embryogenic callus. *Biotechnol* 10:667-74
22. Wang C T Wei Z M (2004) Embryogenesis and regeneration green plantlets from wheat (*Triticum aestivum* L.) leaf base. *Plant Cell Tiss and Org Cult* 77:149-56.
23. Yu Y, Wang J, Zhu M L and Wei Z M (2008) Optimization of mature embryo based high frequency callus inuction and plant regeneration from elite wheat cultivars grown in China. *Plant Breed* 127:249-55.