Tissue Culture Technology is a Breeding Approach in Wheat: An Overview

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Abstract Culturing of plant cells, tissues and organs in controlled and disinfected conditions which includes light intensity, humidity and temperature is known as “Plant Tissue Culture”. From the past several years, regeneration of different plants under lab conditions from different plant cells and tissues has been playing a very important role in examining plant genetics and their physiology. This technique is known to help the plants in propagation and increases their soil’s agronomic performance. In this review, we focus on the impact of plant tissue culture techniques in wheat crop, such as embryo culture, mature and immature embryo culture, effect of media on callus induction and regeneration, media and genotype interaction on callus induction and regeneration, somatic embryogenesis, correlation of agronomic trait on callus induction, and regeneration and somaclonal variations.

Keywords Plant tissue culture; Wheat; Embryo culture; Somatic embryogenesis; Media culture

Introduction
The plant tissue culture includes various techniques used for callus induction, micropropogation, production of secondary metabolites and preservation of plants. Plant tissue culture as a technique of growing explants isolated from the mother plant is a suitable approach to prepare sufficient amount of plant materials within a short span of time in large scale and enhance the natural levels of in vitro production of valuable compounds (Sung, 2006; Pande et al., 2013). It has been also enabled to increase the knowledge in many areas of biology and molecular plant breeding (Pande et al., 2013). The ability of Plant cells and their tissues to a best response in their tissue culture medium and later on their developmental stages can be useful in Agriculture, Horticulture, Plant breeding, Genetic Engineering and in Chemical Industry (Evans et al., 2003). It is great contribution of Plant Tissue Culture in both Agriculture as well as in Ornamental plants. Gottlieb Haberlandt was the first person who became able to culture and isolate different plant cells and tissues in the last phase of the 19th century (Bhojwani and Razdan, 2004).

1 Importance of Tissue Culture in Wheat
An undifferentiated mass of cells obtained during explant culture on different artificial nutrient mediums with growth regulators is called callus. A chemical 2,4-Dichlorophenoxo acetic acid is used for growth regulation during development of callus in cereal tissue culture (naturally synthetic auxin). Naqvi et al. (2002) used 2,4-D with Cytokinins for induction of callus in wheat plants (Mathias et al., 1986ab). It was shown that 2,4-D had different impacts on all genotypes when different concentrations were used (Elwafa and Ismail, 1999). Callogenesis and Organogenesis responses of tissue culture techniques in wheat plant usually depends upon its genotypes, types of ex-plants, physiological status, geographical origin, culture mediums and their interactions (Chen et al., 2006).

2 Role of Explants in In-vitro Regeneration
Callus initiation and regeneration of plantlets in wheat can be held by different types of explants.
1 Mature and immature embryos (Varshney et al., 1999).
2 Coleoptile & Inflorescenses (Benkirane et al., 2000).
3 Seeds (Cure and Mot, 1978; Gosch-Wackerle et al., 1979).
4 Shoot apical meristems (Ahmad et al., 2002).
5 Anthers (Armstrong et al., 1988).

Tissues respond were different during plantlet regeneration on in-vitro conditions (Delporet et al., 2001). Embryos were used for callus culture or DNA delivery techniques in case of wheat plants (Chawla, 2002). Some types of ex-plants showed a vital role in regeneration process used in culture. Regeneration of wheat plant through peduncle was double than regeneration from Rachis plant (Majewska et al., 2007). Most prominent technique to overcome many practical problems is in-vitro culturing of embryo (Dunwell, 1986). Five spring and five winter wheat genotypes were studied by Kim et al., (2011) to develop appropriate regeneration protocol. The more effective genotypes in plantlet formation were spring wheat genotypes as compared with winter wheat genotypes. Optimum developmental stage and embryo length were at 13-14 days and 1.0-1.5 mm respectively to evaluate the regeneration efficiency. Embryos selection with high frequencies of embryogenesis and regeneration capabilities could be more helpful for efficient genetic improvement of wheat plant.

3 Embryo Culture

Embryo culture, an in-vitro technique, is referred as embryo rescue technique used to save deterioration of hybrids. The first mature embryo culture was proposed in 1904 by Norstong. Mature embryos of crucifer were used on a salt medium mixed with sugar. Interspecific hybridization is the specific technique for embryo culture used in plant breeding due to abortion of most of the embryo crosses. Failure of endosperm development is known as the main cause of this abortion (Hu and Wag, 1986) In vitro embryo culture can solve many practical breeding problems to facilitate plant breeders (Dunwell, 1986). Embryo culture may help in the study of nutrition, metabolism and developmental stages of plant. Embryo culture technique can evaluate the effect of plant hormone, growth requirement and environmental conditions on embryo regeneration and embryogenesis (Yeung et al., 1981). This technique can localize the site for germination. In cryopreservation, embryogenesis, germination promoters and technique inhibitors act as a localized site (Grout, 1986). Embryo culture can determine the seed viability and is very useful in assessing the non-ripened and ripened seeds in peach (Tukey, 1944).

4 Mature Embryo Culture in Wheat

Embryos with or without endosperm can be helpful to obtain regeneration of plantlets and induction of embryos (Ozias-Akins and Vasils, 1983 and Zale et al., 2004). Endosperms of mature embryos can also be used for regeneration of plantlets and callus formation (Delporte et al., 2001 and Filippoy et al., 2006). Hanning (1904) obtained viable plant from mature embryos. Experiment was conducted on mineral salt containing medium under aseptic conditions (Norstog, 1979). Genotype response towards regeneration and induction of callus were checked to culture mature embryos (Fazeli-Nasab et al., 2012). By Mature embryo cultures considerable differences in wheat cultivars were found in form of regeneration of plants and effectiveness of callus (Zale et al., 2004). Mature embryos with or without endosperm were used to callus formation and plant regeneration in wheat (Jun-Ying et al., 2006). Embryogenic callus percentage was found more in embryos which are endosperm free (Turhan and Baser, 2004). Mature embryos culture in Triticum aestivum and Triticum durum lead to the development of transgenic plants at a rate 1.28 % to 1.77 % of transformation (Patnaik et al., 2006). It can be used as valuable substitute for immature embryos due to easy access and isolation of mature embryos, (Ding et al., 2009). Mature embryos express lesser variability at their physiological state (Yu et al., 2008).

5 Immature Embryo Culture in Wheat

Callus formation, immature embryo and regeneration of shoot among all explants can produce excellent results (Hou et al., 1997; Arzani and Mirodjahg, 1999; Sarker and Biswas, 2002). Transformation and regeneration in Agro bacterium can be induced by previously cultured embryos callus and immature wheat embryos (Cheng et al., 1997). Immature embryos culture helped in showing wheat callus response towards ABA (Morris et al., 1989). Study of floral developmental mechanism in wheat can be used by immature embryo culture (Bi-Hua et al., 2003).
For callus induction and somatic embryogenesis of cereals, the best ex-plant sources are immature embryos (Wu et al., 2002; Pellegrinreschi et al., 2004). Callogenesis and organogenesis can be easily obtained from immature embryos culture in wheat (Eapen and Rao, 1985; Redway et al., 1990). Best explant for regeneration is immature embryos which can be obtained from callus culture. In case of embryogenesis, the developmental stage of immature embryos is very important (Sears and Deckards, 1982).

6 Effect of Media on Callus Induction and Regeneration in Wheat
Concentration of growth regulators and type of medium can play a vital role in efficient regeneration system in wheat for embryo culture. Raja et al. (2008) proposed that callus induction is better in N6 than MS media. Likewise for regeneration, BAP is far better than kinetin. Exogenous applications of growth regulators can affect the endogenous concentration, control of growth regulators and their biochemical aspects as well. Optimum concentration of 2,4-D can be used for maximum callus induction (Munazir et al., 2010). Regeneration and callus induction can be affected by both auxin and different sugar types. Sugar responses rely on type of auxin to be used (Mendoza and Kaepppler, 2000). In mature and immature culturing of embryos, higher concentration of 4mg/l of 2,4-D is proved to be the best for Callogenesis but this concentration is harmful for regeneration of plants (Khurana et al., 2002). From mature embryos, embryogenic callus formation is best at 1.5 mg/l 2,4-D medium while in case of non-embryogenic callus, quantity of 2,4-D concentration may be increased up to 6 mg/l (Yasmin et al., 2001).

A medium that contains 2,4-D 4 mg/l can produce large callus while maximum number of callus formed at 6 mg/l. Maximum number of developed plants can be achieved at 1 mg/l kinetin concentration. Under lab conditions development of plantlets on M10 medium was excellent and this medium proved best for rooting and propagation of plantlets. Picloram and Dicamba showed healthiest callus production as compared to 2, 4-D. Concentration of these growth regulators is as important as 2, 4-D concentration. Picloram helped in increasing the numbers of shoot growth while when higher concentration of 2, 4-D used, necrotic callus and jair like growth can be observed. Higher concentration of Dicamba does not have significant effect on shoot growth but it may have effect on regeneration. (Parmar et al., 2012) observed different media and cultivars effect on Organogenesis and Callogenesis on wheat. MS media proved best as compared to N6 medium but different cultivars showed different responses to growth regulators at different levels. Genotypes like Inqlab-91 and Lasani-8 respond to a maximum induction of callus (90%) and (78.8%) at 3 mg/l of 2, 4-D concentration. Tata genotype showed 4.43% callus formation at 2 mg/l, Chakwal-97 77.08% at 2.5 mg/l while Khyber and GA-02 expressed 74.30% and 65.97% induction of callus respectively at 3.5 mg/l 2, 4-D. Without 2, 4-D in medium, there is no formation of callus (Rashid et al., 2012). Proliferation and callus induction between different hormones, mostly Auxins are most effective regulators. Callus induction and proliferation in cereals showed maximum response when 2,4-D was used in mediums (Wen et al., 1991). Carbon presence in media acts as an osmotic agent which completes their requirements for energy due to the photosynthetic activity’s reduction under in-vitro conditions. Sugar affected the physiology of cell, its growth and differentiation massively (Gibson, 2000). Carbon source of organogenesis and Callogenesis have to be improved so a reliable source of Carbon should be used for callus induction and regeneration in plants (Mendoza and Kaepppler, 2002). Regeneration of shoot can also be affected by carbon source (Kim et al., 2003).

7 Effect of Gelling Agent on Callus Induction and Regeneration
Different gelling agents can be used for solidification of media which helps embryos on top of culture mediums. In plant tissue culture mostly agar is used as gelling agent (Clapham, 1973; Chu, 1978; Wang and Chen, 1980; Liang et al., 1987). In wheat anther culture, agarose medium has been shown to enhance the embryo induction (Henry et al., 1984; Lucketel et al., 1991). Agarose is very costly but in different studies like wheat (Henry et al., 1984), Barley (Powell et al., 1988 and Mourtizen and Holm, 1992), Maize (Nitsch et al., 1982, Dieu and Beckert, 1986), Rice (Chaleff and Stolarz, 1981) and Rye (Fleishinghaislet et al., 1991), it is proposed that by using agarose medium, induction rate can be increased. Recently different studies showed that agar medium has inhibitory
effects on regeneration of plantlets (Kohlenbach and Wenicke, 1978; Jaramillo and Summers, 1990). Due to High cost and inhibitory effects of agarose, other gelling agents were used (Fadel and Wenzel, 1990). Starch extraction from wheat, barley, rice, maize and potato can be used in comparison with agar (Sorvari, 1987; Riera-Lizarazu and Pienaar and Lesch, 1994) used gelrite medium for solidifying of culture.

8 Effect of Genotype in In-Vitro Planlet-regeneration

Developing a best regeneration system is the prime step in wheat transgenic plant production. In case of wheat plant, different genotypes showed different responses towards tissue culturing. Embryogenic capabilities of genotypes also express different responses to different culture mediums in in-vitro conditions. In the case of plant breeding studies, embryogenic response of genotypes and genetic transformation of wheat are considered as the development of new cultivars for commercial purpose (Vendruscolo et al., 2008). Genotypes had great effect on callus induction, embryogenic formation and plant regeneration (Sears and Deckard, 1982; Mathies and Simpson, 1986; Fennel et al., 1996; Aydin et al., 2011). Ex- plants of Triticum which belong to different genotypes having same age were cultured on medium with same combination of growth regulators. This experiment was conducted for callus formation and regeneration of plantlets. Results showed that callus formation and plantlet regeneration change their course with different genotypes (Arzani and Mirudjagh, 1999; Zale et al., 2004). Similar results were obtained in rice (Hoque and Mansfield, 2004), primula species (Schween and Schwenkel, 2003) and coffee (Molina et al., 2002). Fahmy et al. (2012) conducted an experiment in which they used seven different cultivars on different media to check their regeneration response through somatic embryogenesis. Genotype Giza-164 showed strong performance over all tested cultures.

9 Media and Genotype Interaction on Callus Induction and Regeneration in Wheat

Usually in tissue culturing of wheat, response depends upon genotypes, composition of media and their interaction. Significant interaction was observed for callus induction and plantlet regeneration between phytohormones and genotype. Chakwal-50 performed best (91.25%) at 6 mg/l 2, 4-D while genotype AS-2002 and GA-2002 produced 92.75% and 91.25% callus induction on media consisting 4 mg/l 2, 4-D (Mahmood et al., 2012). Media composition, genotype and their interaction all three had great effect on callus induction and plantlet regeneration. Different types of sugar, Dicamba and genotypes increase the callus formation and regeneration of plantlets (Jiang Ping et al., 2010). By using different growth regulators in media, regeneration capacity can be enhanced. In case of mature embryos, genotypes had strong effects when different growth regulators were used in culture (Nasircilar et al., 2006). Embryogenic ability of callus can be obtained by genetic makeup of genotypes and concentration of exogenous application of harmones (Nawaz et al., 2013).

10 Effect of Culturing Conditions on Callus Induction and Regeneration

In-vitro culture can be affected by different conditions and sources (Caswell et al., 2000; Deplorte et al., 2001). In-vitro plant regeneration in wheat rely on different culturing conditions like callus maintenance and regeneration ability for longer period of time (Domelles et al., 1997). (Naranayasmwamy and Norstog, 1964) obtained that the best temperature for growth of most plant embryos is 25-30℃. Some species like Dhatura did not show response to light intensity during embryo growth (Norstog, 1972). Triticale showed positive effect when red light was expressed on immature embryo regeneration (Angus et al., 1986). For embryo recovery optimum light intensity is 1000 Lux, while temperature is 17-22℃ and 60-65% relative humidity (Campbell et al., 1998; Khan and Ahmed, 2011). Mature culturing in Barley plant showed best response at 22℃, and 55% relative humidity (Hongbo et al., 2005). Lectin levels can be affected by light intensity as it grows 14 times more in light as compared to 10 times growth in dark conditions (Raikhel et al., 1986). To break the seed dormancy, cold treatment at 4℃ is required while some embryos like Lilium need low temperature up to 17℃ (Pierk, 1987).

11 Embryogenic Callus as a Source of Plantlet Regeneration

MackinNon et al. (1987) isolated non-embryogenic callus from embryogenic callus in bread wheat. Callus which was obtained from embryogenic was compact, multicellular and showed off-white to light green colour. This callus was proficient in proliferating to bulbous embryoids, while callus that are dirty whitish, brown or grayish in
color, watery appearance and loose in texture were considered as non-embryogenic callus.

Callus which has embryogenic capacity helps in regeneration of plantlets and can exhibit the capacity of single or many cells to regenerate the whole plant (Gandonu et al., 2005). External morphology of callus differentiated the embryogenic and non-embryogenic behaviors. By increasing the percentage of auxins, embryogenic productions can be decreased. In Inqlab-91 genotype, callus weight and size was increased by this process but the callus was non-embryogenic. Similarly kinetin has negative effect on embryogenic callus formation (Rashid et al., 2009).

12 Somatic Embryogenesis

Embryogenic callus is produced in somatic embryogenesis of somatic cells from which different physiological and morphological changes pass and production of somatic embryos occurs (Quiroz-Figueroa et al., 2006). During in-vitro plant regeneration, somatic embryogenesis showed better response than direct organogenesis (Wang and Bhalla et al., 2004). Conventional breeding program of somatic embryogenesis, cell biological techniques and molecular techniques are proved as valuable tolls to enhance the genetics of different crop plants (Stasolla and Yeung, 2003).

Somatic embryos have different resemblances with zygotic in diverse aspects (Fehar et al., 2003). Morphogenetic procedure of somatic embryogenesis can easily examine the differentiation in cellular and molecular processes (Beneli et al., 2001). This process is taken as the best for germplasms preservation, genetic engineering and artificial seed production (Litz and Gray, 1995; Merkle et al., 1995). By encapsulating somatic embryos, artificial seeds can be easily produced by using a gelling agent (Gosh and Sen, 1994). Different processes of embryogenesis can be studied through this technique. Due to primordial presence of root and shoot, somatic embryos can be easily produced without a need of separate step of rooting (Laux and Jugens, 1997).

13 Correlation of Agronomic Trait on Callus Induction and Regeneration in Wheat

At agronomic trait level, response of different genotypes can be checked towards tissue culture technique. Correlation and regression analysis can be helpful to assess the association between tissue culture and agronomic traits. Culturing of immature embryos and callus regeneration portrayed great differences which expresses the strong effects of genotypes. Callus formation percentage has significant effect with early maturity, peduncle length and its extrusion, spike index and yield/plant, while regeneration capability of callus (CR) and plants production per regenerative embryo (PPE) showed significant correlation with the presence of chlorophyll in flag leaf (Dodig et al., 2008). Five genotypes of wheat plant were mated and resulted crosses were examined for their embryogenic callus potential (EC%) and regeneration percentage of plantlets. Associations between these traits were observed by heading dates and grain yield per plant. Results showed that EC% and PRP% had significant relationship with grain yield per plant (Khaled et al., 2013).

14 Somaclonal Variations in Wheat

Regeneration of plants from embryogenic tissues of somatic cells, showed that variations in morphology and chromosomal changes also occurred during culturing (De Buyser et al., 1988). Immature embryo culturing showed variations in Mt DNA plantlets and these differences quickly stabilized during Callogenesis (Hartmann et al., 1987). Similarly, variations in Mt DNA were also noticed in regeneration of green plants resulting from somatic tissue culture (Aubry et al., 1989). Phenotypic changes showed by plants resulting from in-vitro culturing are the true representatives of genetic variations (Liu and Chen, 1978 a and b; Orton, 1980). Some plants expressed their original morphology and showed that the changes occur during field conditions are due to physiological aspects and not by genetics (Callebaut et al., 1978).

15 Acclimatization of Wheat Plants

Acclimatization of In-vitro developed plantlets is necessary before their transportation to green house. Plantlets at 21°C, low humidity and sixteen hours of continuous photoperiod for two weeks in an environmental chamber, are ready for shifting into green house (Weeks et al., 1993).
16 Conclusion and Future Insights

In summary, Plant Tissue Culture has a great contribution in both agricultural as well as in ornamental plants. In-vitro embryo culture can solve many practical breeding problems to facilitate plant breeding. Embryo culture may help in the study of nutrition, metabolism and developmental stages of plant. Apart from conventional breeding program, somatic embryogenesis, cell biological techniques and molecular techniques have been proved as valuable tools to enhance the genetics of different crop plants. Media composition, genotype and their interaction had all, great effect on callus induction and plantlet regeneration. By using different growth regulators in media, regeneration capacity can be enhanced.

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