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Stimulatory Effect of Thidiazuron Manipulation on Regeneration of Mature Caryopsis Culture in Bread Wheat (*Triticum aestivum* L.) and Pasta Wheat (*Triticum durum* L.)

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Abstract: ***A comparative study was aimed to evaluate the morphogenic response of thidiazuron (TDZ) a novel morphogen, in the Murashige and Skoog (MS) nutrient medium on mature caryopsis culture of hexaploid bread wheat (Triticum aestivum L. cv. HD2329) and tetraploid pasta wheat (Triticum durum L. cv. PDW215). To begin with, mature caryopsis exhibits slow and inhibited germination followed by semi-compact callus formation from the basal region of growing coleoptiles on nutrient medium supplemented with 2, 4-dichlorophenoxyacetic acid (2,4-D) alone or in combination with either 6-benzylaminopurine (BAP) or TDZ. In contrary, caryopses cultured on either BAP or TDZ alone supplemented medium, show direct multiple-shoot regeneration instead of callusing from the base of developing coleoptiles. Furthermore, results indicate that mature caryopses of both wheat varieties show significant improvements in frequency of multiple-shoot regeneration (53%) and (39%) respectively on manipulation of TDZ concentration in combination with BAP. Thus, TDZ proves to be stimulatory in terms of multiple-shoot regeneration from caryopsis culture. However, other treatments such as 2,4-D either with TDZ or BAP reflected very weak combinations and failed to contribute any effective roles in regeneration of both wheat varieties. Hence, the results involving the stimulatory response of TDZ for mature caryopsis regeneration in wheat suggest that further investigations are required to understand the molecular involvement of TDZ during cellular metabolic pathways and its interaction with BAP during in vitro cell regeneration.***

Keywords: Wheat, Caryopsis, TDZ, Callus, Shoot Regeneration

1. Introduction

Wheat is one of the most primary sources of food and nutrition for human populations all over the world and it is well studied that almost 95% of the cultivated wheat *T. aestivum* (hexaploid) type is used mainly for the preparation of bread and other baked products whereas the remaining 5% of *T. durum* (tetraploid) type wheat that is essentially used for making pasta and macaroni. Development of efficient plant regeneration protocol from either single cell or organized tissue is important for many commercially important crops like wheat the common source of energy and proteins for the world population (Kheyrodin and Kheyrodin, 2014).

Moreover, in addition to conventional breeding technique, application of gene transformation technology has been proved an alternative approach to improve the quality and quantity of wheat crops. Significantly, the success of transgenic wheat production depends on the establishment of efficient and stable plant regeneration system but the reproducible and dependable regeneration protocols suitable for various explants and genotype is lacking in wheat. Hence, it proves to be major limitations for implementation of an effective wheat functional genomics programme (Bhalla, 2006; Ganeshan *et al*., 2006; Vasil, 2007; Chauhan *et al*., 2007).

In general, immature embryonic tissues have been proved more competent tissue in terms of regeneration potential as compared to mature embryonic tissues in graminaceous crops but additionally other tissues such as non-endosperm supported mature embryos directly from seeds (Ozias-Akins and Vasil, 1983; Kato *et al.,* 1991; Kintzios *et al.,* 1996; Varshney *et al.,* 1999; Mendoza and Kaeppler, 2002; Li *et al.,* 2003; Patnaik and Khurana, 2003; Zale *et al.,* 2004), thin pieces of mature embryos (Delporte *et al.,* 2001) and endosperm supported mature embryos (Ozgen *et al.,* 1996, 1998; Chen *et al.,* 2006; Filippov *et al.,* 2006) have been also used for callus formation and plant regeneration (Aydin *et al.,* 2011). Unfortunately, immature embryogenic tissues are restricted only for a short-period in plant life; therefore, procurement of these tissues seems to be quite tedious. Hence, mature tissues like mature embryo and caryopses have enough potential to be treated as immediate possible alternative for the studies on regeneration and transformation of wheat.

During the last few decades, several compounds have been synthesized and also employed to identify for inducing the regeneration potential in plant cell (Murthy et al., 1998) and morever, the morpho-regulatory potential of TDZ was studied that led to its application as an effective bioregulant in cell and tissue cultures in many plant species (Li *et al.,* 2000; Hosseini-Nasr and Rashid, 2000; Svetla *et al.,* 2003; Matand and Prakash, 2007; review Guo *et al.,* 2011). Additionally, TDZ is known to induce not only adventitious and/or axillary shoot production through organogenesis, but also somatic embryogenesis in many dicot plant species (Murthy *et al.,* 1998). Most studies involving the morpho-

regulatory effects of TDZ in plant during *in vitro* culture are mainly based on dicot plant species whereas the literatures involving the effects of TDZ in wheat regeneration are very limited. The present study thus involves the application of TDZ during *in vitro* culture of mature caryopsis of Indian bread wheat and pasta wheat and also to understand its morphoregulatory response in wheat regeneration.

2. Materials and Methods

Collection and Preparation of Experiment Materials

To begin with, mature seeds of *Triticum aestivum* cv. HD2329 and *Triticum durum* cv. PDW215 were obtained from IARI, New Delhi, India and dry seeds of both wheat varieties were initially washed in running tap water with Teepol (Reurthckit and Colman India Ltd.) and then surface sterilized with ethanol (95%) for 30 seconds followed by mercuric chloride (0.1%) treatment for 5 minutes in a laminar flow. Mature caryopses were further continuously washed with sterile distilled water (SDW) for another 4-5 minutes followed by air drying on sterilized tissue paper in a laminar flow to minimize the water-borne contamination of explants. Finally, sterilized mature caryopses were inoculated on nutrient media by placing embryonic-axis of the caryopsis away from the contact of nutrient medium.

Nutrient Medium Composition

Nutrient medium comprised of MS (Murashige and Skoog, 1962) supplemented with 20 g/l sucrose, 100 mg/l myo-inositol and was solidified with 0.8% agar. Nutrient media supplemented with various concentrations of plant growth regulators either alone or in combinations were used (Table 1) to study on regeneration of both wheat varieties.

Table 1: Composition of Nutrient medium with or without Plant Growth Regulators

Nutrient Medium	*2,4-D (µM)*	*BAP (µM)*	*TDZ (µM)*
M0	-	-	-
M1	5	-	-
M2	10	-	-
M3	20	-	-
M4	-	5	-
M5	-	10	-
M6	-	20	-
M7	-	-	5
M8	-	-	10
M9	-	-	20
M10	-	5	5
M11	-	5	10
M12	-	5	20
M13	5	5	-
M14	5	10	-
M15	5	20	-
M16	5	-	5
M17	5	-	10
M18	5	-	20

Explants were sub-cultured after every 15 days and at the end of four weeks of culture initiation, actively growing multiple-shoots were excised from the main explants and then were transferred on MS-basal medium for further growth of regenerated shoots.

Culture Conditions

The explants inoculated cultures were incubated for 10 days in dark at 26 ± 2^0C, and were further maintained at 26 ± 2^0C under 16 h photoperiod with a light intensity of 100–125 mmoles $m^{-2}s^{-1}$ provided by fluorescent tube light (Philips India Ltd.)

Data Analysis

Each experiment was performed with 25 explants per treatment and three replicates were maintained for each treatment and data analysis was done by using three individual experiments. Further, for analyzing the results, observations on frequency of explants showing callus formation or regeneration of shoots was recorded after 5-6 weeks of culture initiation.

3. Results and Discussions

In general, *in vitro* regeneration of plants depends on various controlling factors but the type of explants, the genotype and composition of nutrient media are some of the important factors affecting *in vitro* wheat tissue culture (Gill *et al*., 2014). A phenylurea substitute, thidiazuron (TDZ) was employed first time as a cotton defoliant (Arndt *et al*., 1976) and it was later found that TDZ showed high cytokinin activity in promoting growth of cytokinin-dependent callus cultures (Mok *et al*., 1982). Probably, TDZ may stimulate conversion of cytokinin nucleotides to more biologically active nucleosides (Capelle *et al*., 1983) and/or stimulate accumulation of endogenous purine cytokinins (Thomas and Katterman, 1986).

Previous studies indicate that TDZ is known an efficacious regulator of *in vitro* morphogenesis of many dicot plants, influencing callusing, shoots regeneration, somatic embryogenesis, and protoplast division (Khurana *et al.*, 2005). However, its applications to cereal tissue culture have been limited (Shan *et al.*, 2000; Vikrant and Rashid 2002; Gairi and Rashid 2004; Sharma *et al*., 2004, 2005; Ganeshan *et al*., 2006). Thus, the present investigation aims to explore the morpho-regulatory action of TDZ on mature caryopsis culture in two Indian wheat; *T. aestivum* and *T.durum* and also investigate the stimulatory response if any of TDZ on regeneration by optimizing both the concentration and combination of TDZ with other plant growth regulators.

Effect of 2, 4-D

In a comparative study of mature caryopsis culture on 2,4-D free basal medium (M_0), caryopses exhibit germination followed by development into normal seedlings (**Figure 1A & B**) in both wheat varieties while on culture to 2,4-D media (M_1, M_2 and M_3), caryopses showed relatively inhibited germination followed by the induction of callus in both wheat varieties from the basal regions of the developing young seedlings (**Figure 1C & D**) within 4 weeks of culture initiation. The frequency of callus forming explants in both wheat varieties was recorded to be variable and in case of *T. aestivum*, the maximum frequency (52%) explants (**Figure 2**) exhibit callus formation at high 2,4-D (10µM-M_2) whereas in *T. durum*, frequency of callus forming cultures was found to be maximum (34%) at very high concentration of 2,4-D (20µM-M_3) supplemented nutrient medium.

Generally, calli induced at 2,4-D (10µM) supplemented medium were found to be semi-compact and on transfer to 2,4-D-free basal nutrient medium, these calli appeared to be nodular but failed to show somatic embryogenesis. Significantly, in rice caryopsis culture, callus induced at 2,4-D (10µM) exhibits nodular appearance and differentiates further into somatic embryos on transfer to 2,4-D-free nutrient medium (Vikrant *et al.*, 2012). Moreover, callus formed at high 2, 4-D (20µM) supplemented medium appeared to be friable in nature and further necrosed without differentiation into shoots/roots or embryo.

Figure 1- (A-D)

A) In three-week-old culture of *T. aestivum*, mature caryopsis germinates and develops into a seedling on MS-Basal medium.

B) In five-week-old culture of *T. durum*, mature caryopsis shows the germination and development into a seedling on MS-Basal medium.

C) One-week-old culture of *T. aestivum*, caryopsis exhibits callus formation at the seedling base on MS+2,4-D (5µM) nutrient medium.

D) One-week-old culture of *T. durum*, caryopsis exhibits callus formation from the basal region of growing seedling on MS+2,4-D (5µM) nutrient medium.

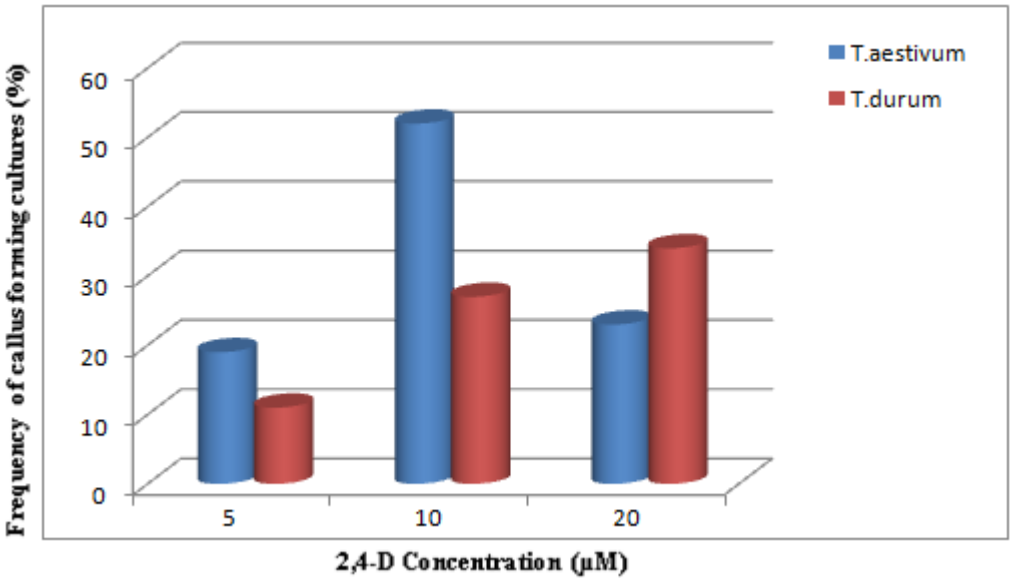

Figure 2: Frequency of cultures showing callus formation on various concentrations of 2, 4-D in caryopsis culture of *T. aestivum* and *T. durum*.

Effect of BAP

On BAP supplemented nutrient media (M_4, M_5 and M_6), caryopses germinated normally like control experiments, however, in one-month–old culture, seedling started to show gradual necrosis followed by induction of multiple shoots from the basal region of the seedling (**Figure 1E**). However, there was variations in terms of multiple-shoots regeneration frequency and moreover, number of shoots/explants was dependent on the concentration of BAP in the nutrient medium. In *T. aestivum*, the maximum frequency (19%) of explants showing multiple-shoots regeneration was observed at high concentration (10µM-M_5) and further increase in BAP concentration (M_6) could not be effective to support multiple-shoot regeneration. However, in *T. durum*, BAP at

low concentration (5µM-M_4) exhibited the maximum frequency of multiple-shoots regeneration from the developing seedlings (**Figure 1F**) and almost 21% explants exhibited multiple-shoots regeneration (**Figure 3**). In both wheat varieties, BAP at high concentration (20µM- M_6) failed to prove effective for the regeneration.

Figure 1 (E-F)

E) Four-week-old caryopsis culture on MS+BAP (5µM) shows induction of multiple-shoots from the basal region of growing seedling in *T. aestivum.*

F) Four-week-old caryopsis culture on MS+BAP (5µM) shows multiple-shoot formation from the seedling base in *T. durum.*

Effect of TDZ

During this study in both the wheat varieties, explants cultured at higher TDZ concentrations (10µM- M_8) and (20µM- M_9) exhibit slow seedling elongation and even after subcultures, callus formation was not seen at the basal region of the seedlings. Similar results involving absence of callusing on TDZ alone nutrient medium have also been observed in previous studies (Shan *et al*., 2000; Vikrant and Rashid 2002). However, similar to BAP treatments, supplementation of TDZ in nutrient MS-medium supports the multiple-shoots regeneration directly from the basal region of the seedling and frequency of cultures showing shoots regeneration as well as number of shoots per explants was found to be higher in pasta wheat then bread wheat.

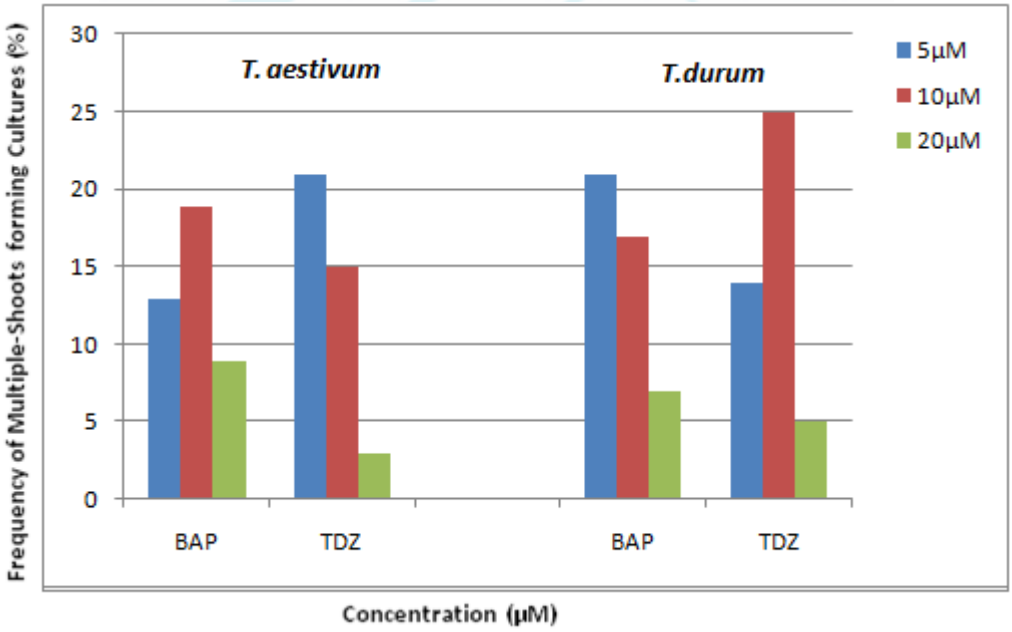

Figure 3: Frequency of cultures showing Multiple-shoots regeneration from caryopsis culture on medium supplemented with various concentrations of BAP and TDZ in *T. aestivum* and *T .durum.*

Significantly, in case of *T. aestivum*, the maximum frequency of explants regeneration (21%) in terms of direct multiple-shoots formation **(Figure 1G & H)** was observed at low concentration of TDZ (5µM-M_7) supplemented medium and further increase in TDZ concentration **(Figure 1I)**, proved to be little effective for regeneration. However, in case of *T. durum*, the maximum frequency of regeneration (25%) was recorded at relatively high level of TDZ (10µM-M_8) containing nutrient medium (**Figure-3**). The morphogenic effect of TDZ in terms of frequency of cultures showing multiple-shoots regeneration as well as number of shoots/explants was found to be relatively higher in *T. durum* (**Figure 1J**) than *T. aestivum* wheat. Furthermore, explants cultured on very high concentrations of TDZ (20µM-M_9) for both wheat varieties, regeneration frequency was found to be less than the frequency obtained at BAP of the same concentration (M_6)

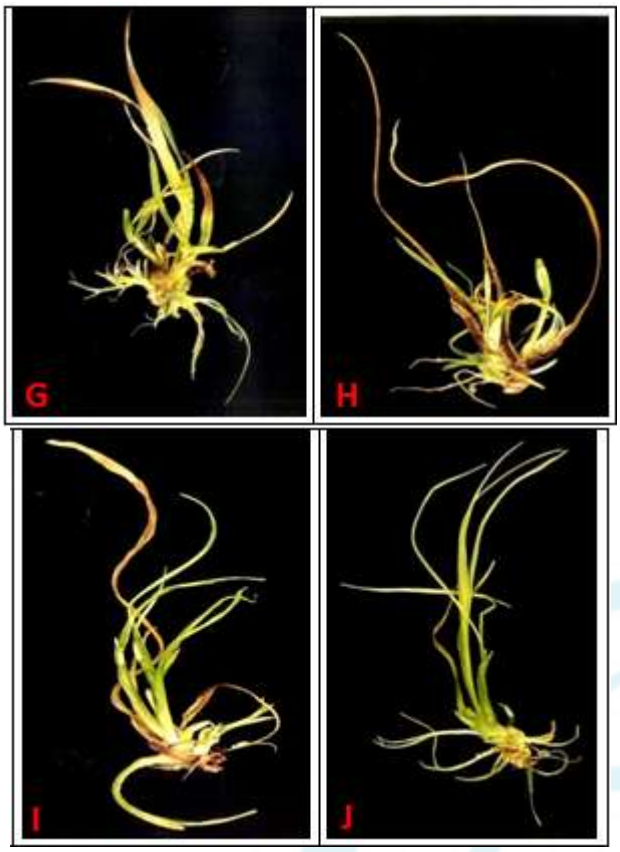

Figure 1-(G-J)

G) Three-week-old caryopsis culture on MS+TDZ (5µM) exhibits induction of multiple-shoots from the seedling base in *T. aestivum*.

H) Four-week-old caryopsis culture on MS+TDZ (5µM) shows formation of multiple-shoots from the basal region of growing seedling in *T. aestivum.*

I) Four-week-old caryopsis culture on MS+TDZ (10µM) exhibits induction of multiple-shoots from the seedling base in *T. aestivum.*

J) Three-week-old caryopsis culture on MS+TDZ (10µM) exhibits multiple-shoots formation from the basal region of growing seedling in *T.durum*.

In a previous experiment, spring wheat (Bob White) variety exhibits good regeneration ability at TDZ (0.9 mM) and was later commonly used in wheat transformation experiments (Weeks *et al*., 1993; Vasil *et al.,* 1993). Of late, in a study on mature grains culture of two bread wheat varieties viz. (HD2967 and Bobwhite), highest shoot regeneration from mature grain derived calli was observed on MS medium supplemented with TDZ (0.3 mg/l) in HD 2967 whereas in Bobwhite variety, the optimum regeneration was recorded at TDZ (0.2 mg/1) and moreover, TDZ was found to be better morphogen in terms of regeneration over BAP and IAA combinations (Gill *et al.,* 2014)

Effect of BAP with 2, 4-D

In order to understand the effect of various concentrations of BAP (5, 10 and 20µM) in combination with 2,4-D (5µM) on caryopses culture, caryopses which were inoculated at 2,4-D (5µM) with BAP (10µM-M_{14}) medium, germinated slowly and developed very little friable callus from the basal region of developing seedling in 3-weeks of culture initiation and only 2% cultures showed differentiation of shoots in *T. aestivum* whereas in case of *T. durum*, regeneration of shoots was seen in 4% cultures at medium supplemented with equimolar concentrations of 2,4-D and BAP (5µM-M_{13}). During this study, other combinations of 2, 4-D and BAP (M_{15}) could not be functional for shoots regeneration in both wheat varieties (**Figure 4**).

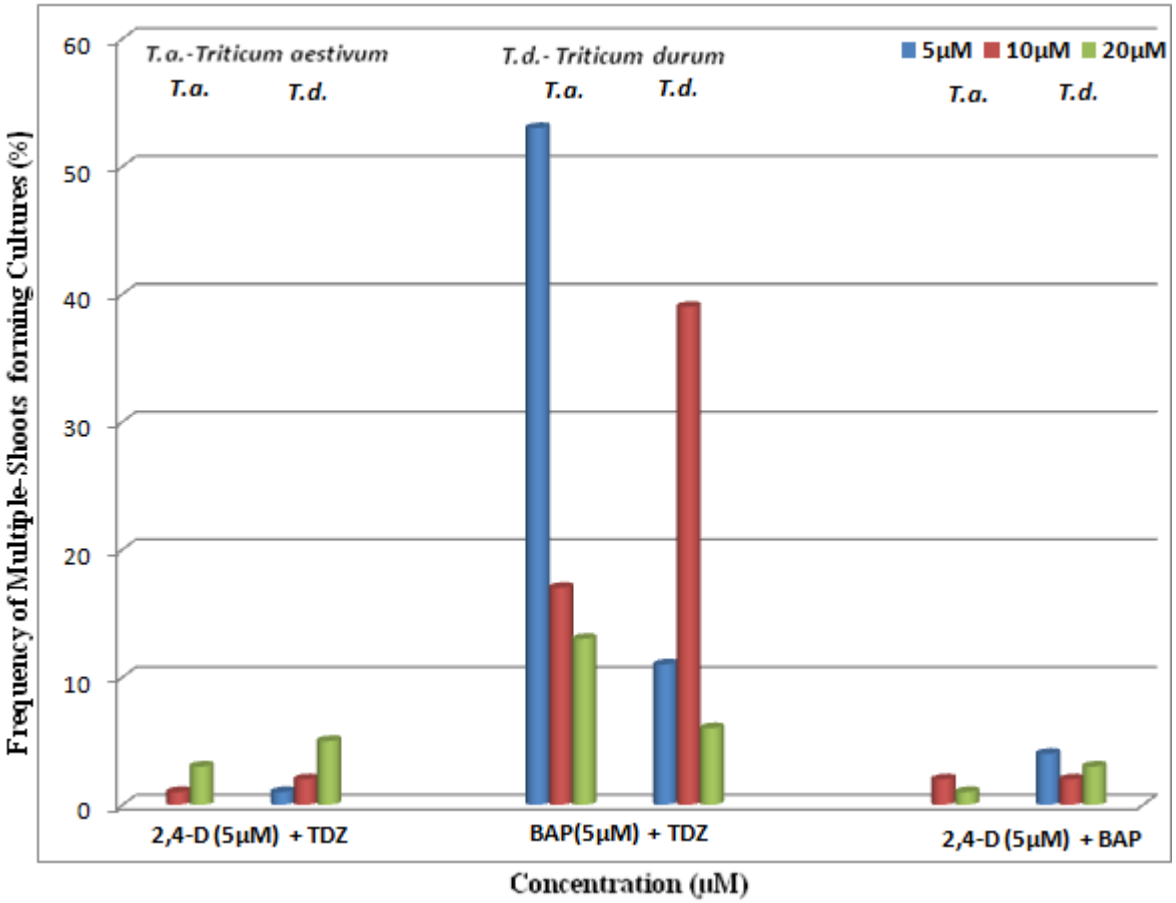

Figure 4: Frequency of cultures showing Multiple-shoots regeneration from caryopsis on medium supplemented with various concentrations of TDZ or BAP with 2,4-D and TDZ with BAP together in *T. aestivum* and *T.durum*.

In general, the results of the present study are in contrast of earlier reports where MS medium containing BAP (2.0 mg/l) in combination with IAA (1.0 mg/l) and also BAP (1.0 mg/l) in combination with 2,4-D (0.5 mg/l) were proved to be very effective for shoots regeneration (Lu, 1993; Mohmand, 1994). However, the application of BA during subculture significantly promotes embryogenic callus formation in wheat mature embryos culture (Yu *et al.*, 2008).

Morphogenic Effect of TDZ with 2,4-D

In contrast to the *in vitro* culture of dicot plants, where TDZ alone is sufficient to enhance regeneration, in monocots, TDZ works well when used in combination with other growth regulators (Shan *et al.*, 2000; Gairi and Rashid 2004; Sharma *et al.*, 2004, 2005). In barley, the combination of picloram (2 mg/l) and TDZ (3 mg/l) has been found to be suitable for direct multiple-shoot regeneration, but not for callusing (Sharma *et al.*, 2004) and further study also indicates that such combination proves to be more suitable for direct shoot regeneration in two winter wheat genotypes (Sharma *et al.*, 2005).

Various concentrations of TDZ (5, 10 and 20 µM) with 2,4-D (5µM) was tested during present study and in both wheat varieties explants which were inoculated at 2,4-D (5µM) with High TDZ (20µM-M_{18}) could show shoot regeneration very poorly from the induced friable callus in 3% and 5% cultures respectively. However, other combinations of 2,4-D with TDZ (M_{16} and M_{17}) could not be effective in terms of regeneration for both wheat varieties. In a previous study, the effects of the TDZ level (0.2 mg/l) on wheat regeneration of the two varieties (Bob White and Hi-Line) were compared to those of other plant growth regulators and combinations commonly used for wheat regeneration. In both varieties, TDZ resulted in the highest mean percentage regeneration. The combination of Bob White and TDZ was superior to any other combinations used whereas in Hi-Line, no significant difference was found between TDZ, 2,4-D and kinetin plus NAA treatments, however, all these treatments performed significantly better than dicamba (Shan *et al.*, 2000).

Present study suggests that BAP or TDZ alone is competent enough to support direct multiple-shoots regeneration and frequency of regeneration was recorded even more (**Figure 3**) than the frequency obtained in combination of BAP or TDZ with auxin 2,4-D (**Figure-4**). This is in conformity with previous studies where TDZ alone in the subculture medium favors plant regeneration and even more than if TDZ is supplemented in combination with the auxins in the subculture nutrient medium (Tian *et al.*, 1994; Anju *et al.*, 2003). Moreover, in a recent study on *T. aestivum*, considerable improvement in the regeneration frequency was recorded with a combination of TDZ and 2,4-D (Parmar *et al.*, 2012). However, present study suggests that 2,4-D alone is able to generate a semi-compact callus but addition of TDZ along with 2,4-D during callus induction medium proved to be very little effective for shoot regeneration.

4. Stimulatory Effects of TDZ on Regeneration

Previous studies reveal that auxin and cytokinin combinations generally improve the regeneration frequencies (Bhaskaran and Smith, 1990; Pola *et al.*, 2008). In monocotyledons species, it is established that TDZ induces multiple-shoot formation and also promoting callus regeneration. It is suggested that TDZ has potential for enhancing the regeneration of cereal and grass species (Shan *et al.*, 2000) and regeneration of multiple-shoots has been observed using 10µM TDZ in rice (Gairi and Rashid, 2004). In sorghum also, it is observed that the use of a combination of BAP, TDZ and IAA could enhance multiple-shoot production (Pola *et al.*, 2008; Al-Saied *et al*., 2014).

Furthermore, the significance of TDZ was analyzed in a variety of experimental approaches in small grain cereals to induce multiple-shoots formation and also to establish *in vitro* regeneration protocols (Schulze, 2007). During present study, low concentration of growth hormone BAP was employed along with various concentrations of TDZ that led to a significant increase in the regeneration frequency of caryopses explants. With respect to regeneration, both the varieties of wheat were tested and found to be differed in terms of frequency of explants exhibiting multiple-shoots regeneration or number of shoots/explants. Moreover, the maximum regeneration frequency was observed in medium carrying BAP (5µM) in combination with TDZ (5µM) for bread wheat varieties (**Figure 1K, L & M**) whereas in case of *T. durum*, TDZ at relatively higher concentration (10µM) along with BAP (5µM) could be proved effective for stimulation in shoots regeneration (**Figure 1 N & O**). In *T. aestivum*, the stimulatory effect of TDZ in terms of frequency of cultures indicating direct multiple-shoot regeneration was (53%) whereas in *T. durum*. the enhancing response of TDZ was recorded in (39%) cultures (**Figure 4**).

Figure 1 (K-M)

K) Four-week-old caryopsis culture on MS+BAP (5µM) + TDZ (5µM) shows multiple-shoots formation from the basal region of growing seedling in *T. aestivum*.

L) Five-week-old caryopsis culture on MS+BAP (5µM) + TDZ (5µM) exhibits induction of multiple-shoots from the seedling base in *T. aestivum*.

M) Six-week-old caryopsis culture on MS+BAP (5µM) + TDZ (5µM) exhibits development of multiple-shoots from the seedling base in *T. aestivum*.

Figure 1 (N-O)

N) Four-week-old caryopsis culture on MS+BAP (5µM) + TDZ (5µM) shows multiple-shoots formation from the seedling base in *T. durum*.

O) Five-week-old caryopsis culture on MS+BAP (5µM) + TDZ (10µM) exhibits formation of multiple-shoots from the basal region of growing seedling in *T. durum*.

Furthermore, low concentration of TDZ (5µM) along with low concentration of BAP (5µM) has been proved highly effective combination to promote explant potentials for direct multiple–shoots regeneration in *T. aestivum*. However, previous study on various wheat genotypes reveals that TDZ in lower concentration supports callusing in combination with auxin for immature inflorescence and embryo culture whereas TDZ proves to be effective in combination with cytokinin during the regeneration phase in mature embryo culture (Chauhan *et al.*, 2007). Earlier report (Ganeshan *et al.*, 2006) also reveals the stimulatory response of TDZ on durum wheat mature embryo culture, where the most number of shoots/explant was obtained on culture medium containing TDZ (4.5µM) and BAP (4.4 µM) while on TDZ alone, shoot regeneration frequency was found to be less as obtained in present study also. Moreover, the regeneration frequency from the three winter wheat genotypes was found to be highest on culture medium containing TDZ (9.1µM) and BAP (4.4 µM).

Additionally, other plant growth regulators alone or in combinations have been studied in *T. aestivum*, TDZ alone exhibits the better regeneration response than other

treatments tested and the highest percentage of the shoots formation was recorded in comparison to BA and KN (Almobasher and Ahmed, 2015). More recently, TDZ was proved to be more effective than BA and Zeatin, for the regulation of morphogenesis; particularly for shoot formation in *T. monococcum*, (Miroshnichenko *et al.*, 2017). Moreover, in *T. aestivum*, regardless of embryo source or genotypes, TDZ was found to exert a positive effect on the plant regeneration process in immature, mature and endosperm-supported mature embryos of five different genotypes (Benlioğlu and Avci birsin, 2017).

Interestingly, the high concentration of TDZ (20µM) with low BAP (5µM) was the least effective combination during present study for the stimulation of multiple-shoots regeneration compared to other combinations of BAP and TDZ in both wheat varieties, indicating that TDZ at very high concentration is not suitable for promoting the regeneration. Moreover, the stimulatory response of TDZ for shoot-regeneration in caryopsis culture was found to be more evident in *T. aestivum* than *T. durum*. Significantly, present study reveals that TDZ alone or in combination with BAP could be used for *in vitro* regeneration of the mature caryopses in wheat cereals.

In general, it is established that TDZ supports for direct shoot regeneration in monocots and present study also indicates that both the concentration and combination of TDZ application are critical for efficient wheat regeneration and a balanced exposure is needed for the regeneration of mature caryopses explants. Moreover, the key limiting factor in the successful generation of transgenic plants is the optimization of conditions for regeneration of plantlets after several rounds of selection (Patnaik *et al.*, 2006). This protocol based on stimulatory behavior of TDZ for wheat regeneration could be used for producing transgenic bread and pasta wheat varieties. Further, it offers a significant scope to understand more about the metabolic interactions of TDZ alone or along with BAP at cellular level.

5. Conclusion

Although TDZ is applied to *in vitro* regeneration of many dicot species but very little information is available regarding its effects on *in vitro* regeneration of cereal and other monocot species. Present study has demonstrated that mature caryopses are viable alternative to immature tissue derived explants for efficient shoot regeneration in a comparatively genotype independent manner on a simple TDZ or with BAP containing MS-basal nutrient media.

Results indicate that caryopses cultured on either BAP or TDZ alone supplemented medium, induce direct multiple-shoots regeneration in both wheat varieties *T. aestivum* and *T. durum*. However, TDZ in combination with BAP proves to be stimulatory in terms of multiple-shoot regeneration in both wheat varieties but at different concentrations. Further, it also implies that TDZ could be treated as a novel morphogen to obtain the high regeneration frequency during mature caryopsis or embryo culture in other cereal and grass species.

6. Acknowledgments

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References

[1] Almobasher H.A., and Ahmed M.M. (2015). Effect of plant growth regulators on micropropagation and rooting of the regenerated plant-lets derived from wheat (*Triticum aestivum* L.) embryogenic callus. International Journal of Development Research. Vol. 5 (12): 6209-6212.

[2] Al-Saied H., l-Beskii F., and Askoul I. (2014). Standardization of Tissue Culture Protocols for Callus Induction and Plant Regeneration from Mature Embryo of Sorghum [Sorghum bicolor L. Moench]. International Journal of Chem.Tech. Research, Vol.6 (5): 2710-2718.

[3] Anju S., Uppal S., Sudhir S. and Sharma S.K. (2003). An alternative source for regenerable embryogenic callus induction from shoot tips of wheat (*Triticum aestivum* L.). Indian Journal of Genet and Plant Breeding .63:209-211.

[4] Arndt F.R., Rusch R., Stillfried H.V., Hanisch B. and Martin W.C. (1976). A new cotton defoliant. Plant Physiol. 57: 99.

[5] Aydin M., Tosun M. and Haliloglu K.. (2011). Plant regeneration in wheat mature embryo culture. African Journal of Biotechnology Vol. 10(70):15749-15755.

[6] Benlioğlu B. and Avci Birsin M. (2017). A thidizuran (TDZ) – based efficient plant regeneration system. Ciência e Técnica Vitivinícola. Vol. 32 ((11): 108-119.

[7] Bhalla P.L. (2006). Genetic engineering of wheat—current challenges and opportunities. Trends Biotechnol. 24: 305–311.

[8] Bhaskaran S. and Smith R. H. (1990). Regeneration in cereal tissue culture: A review. Crop Sci. 30: 1328-1336.

[9] Capelle S.C., Mok D.W.S., Kirchner S.C. and Mok M.C. (1983). Effects of thidiazuron on cytokinin autonomy and the metabolism of N6-(Y2- isopentyl) [8-14c] adenosine in callus tissues of *Phaseolu lunatus* L.Plant Physiol. 73: 796-802.

[10] Chauhan H., Desai. S. A. and Khurana, P. (2007). Comparative analysis of the differential regeneration response of various genotypes of *Triticum aestivum*, *Triticum durum* and *Triticum dicoccum*. Plant Cell, Tissue and Organ Culture, 91(3): 191-199.

[11] Chen J.Y., Yue R.Q., Xu H.X. and Chen X.j. (2006). Study on plant regeneration of wheat mature embryo under endosperm- supported culture. Agric. Sci. China. 5: 572-578.

[12] Delporte F., Mostade O. and Jacquemin J.M. (2001). Plant regeneration through callus initiation from thin mature embryo fragments of wheat. Plant Cell, Tissue and Organ Cult. 67: 73-80.

[13] Filippov M., Miroshnichenko D., Vernikovskaya D. and Dolgov S. (2006). The effect of auxin and exposure to auxin and genotypes on somatic embryogenesis from mature embryos of wheat. Plant Cell, Tissue and Organ Cult. 84: 213-222.

[14] Gairi A. and Rashid A. (2004). TDZ-induced somatic embryogenesis in non-responsive caryopses of rice using a short treatment with 2,4-D. Plant Cell Tiss Org Cult. 76:29–33.
[15] Ganeshan S., Chodaparambil S.V., Baga M., Fowler D.B., Huel P., Rossnagel B. and Chibber R.N. (2006). *In vitro* regeneration of cereals based on multiple shoot induction from mature embryos in response to thidiazuron. Plant Cell Tiss Org Cult. 86:63–73.
[16] Gill A.K., Gosal S.S. and Sah S.K. (2014). Differential cultural responses of wheat (*Triticum aestivum* l.) with different explants. Journal of Cell and Tissue Research. Vol. 14(2): 4351-4356.
[17] Guo B., Abbasi B.H., Zeb A., Xu L.L. and Wei Y. H. (2011). Thidiazuron: A multi-dimensional plant growth Regulator, African Journal of Biotechnology Vol. 10 (45): 8984-9000.
[18] Hosseini-Nasr M. and Rashid A. (2000). Thidiazuron-induced shoot-bud formation on root segments of *Albizzia julibrissin* is an apex controlled, light-independent and calcium-mediated response. Plant Growth Regul. 36: 81-85.
[19] Kato K., Chowdhury S.H. and Harada S. (1991). Effect of culture condition on plant regeneration capacity of mature embryo derived callus in wheat (*Triticum aestivum* L.). Wheat Inf Serv. 72: 95-97.
[20] Kheyrodin H. and Kheyrodin S. (2014). Study of Plant Tissue Culture Technology. .J. Biol. Chem. Research. 31(2):1236-1244.
[21] Khurana P., Bhatnagar S. and Kumari S. (2005). Thidiazuron and woody plant tissue culture. J Plant Biol, 32:1–12.
[22] Kintzios S.E., Trantafyllou M. and Drossopoulos J. (1996). Effect of genotype and different growth regulator treatments on callus induction, proliferation and plant regeneration from mature wheat embryos. Cereal Res. Commun. 24(2): 147-153.
[23] Li H., Murch S.J. and Saxena P.K. (2000). Thidiazuron-induced de novo shoot organogenesis on seedlings, etiolated hypocotyls and stem segments of Huang-qin. Plant Cell Tissue Organ Cult. 62: 169-173.
[24] Li W., Ding C.H., Hu Z., Lu W. and Guo G.Q. (2003). Relationship between tissue culture and agronomic traits of spring wheat. Plant Sci. 164: 1079-1085.
[25] Lu, C.Y. (1993). The use of Thidiazuron in tissue culture. In vitro Cell Dev. Bio. 29(2): 92-96.
[26] Matand K. and Prakash C.S. (2007). Evaluation of peanut genotypes for in vitro plant regeneration using thidiazuron. J. Biotechnol. 130: 202-20.7
[27] Mendoza M.G. and Kaeppler H.F. (2002). Auxin and sugar effects on callus induction and plant regeneration frequencies from mature embryos of Wheat (*Triticum aestivum).* In vitro Cell. Dev. Biol. 38: 39-45.
[28] Miroshnichenko D., Chaban I., Chernobrovkina, M. and Dolgov, S. (2017). Protocol for efficient regulation of *in vitro* morphogenesis in einkorn (*Triticum monococcum* L.), a recalcitrant diploid wheat species. PLOS ONE. DOI:10.1371/journal.pone.0173533.
[29] Mohmand A.S. (1994). Induced variability for some agronomic and morphological characters in wheat. Pak. J. Agric. Res., 15(1): 100-107.
[30] Mok M.C., Mok D.W.S., Armstrong D.J., Shudo K., Isogai Y. and Okamoto T. (1982). Cytokinin activity of N-phenyl-N_-1,2,3-thidiazol-5-yl urea (Thidiazuron). Phytochemistry. 21:1509-1511.
[31] Murashige T. and Skoog F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15: 473–497.
[32] Murthy B.N.S., Murch S.J. and Saxena P.K. (1998). Thidiazuron: A potent regulator of in vitro plant morphogenesis. In Vitro Cell Dev. Biol. Plant. 34: 267-275.
[33] Ozgen M., Turet M., Ozcan S. and Sancak C. (1996). Callus induction and plant regeneration from immature and mature embryos of winter durum wheat genotypes. Plant Breed. 115: 455-458.
[34] Ozgen M., Turet M., Altinok S. and Sancak C. (1998). Efficient callus induction and plant regeneration from mature embryo culture of winter wheat genotypes. Plant Cell Reports. 18: 331-335.
[35] Ozias-Akins P. and Vasil I.K. (1983). Callus induction and growth from the mature embryo of wheat. Protoplasma. 115: 104-113.
[36] 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 Biol Plants. 18(2):177–183.
[37] Patnaik D. and Khurana P. (2003). Genetic transformation of indian bread (*Triticum aestivum)* and pasta (*Triticum durum)* wheat by particle bombardment of mature embryo-drived cali. BMC plant biol. 3(5): 1-11.
[38] Patnaik D., Vishnudasan D. and Khurana P .(2006). Agrobacterium— mediated transformation of mature embryos of *Triticum aestivum* and *Triticum durum*. Current Science, 91:307–317.
[39] Pola S., Mani N.S. and Ramana T. (2008). Plant tissue culture studies in Sorghum bicolor: immature embryo explants as the source material. Int. J. Plant Prod. 2:1-14.
[40] Schulze J. (2007). Improvements in cereal tissue culture by Thidiazuron: a review. Fruit Veg Cereal Sci Biotechnol. 1(2): 64-79.
[41] Shan X.Y., Li D.S. and Qu R.D. (2000) Thidiazuron promotes in vitro regeneration of wheat and barley. In Vitro Cell. Dev. Biol.-Plant. 36: 207–210.
[42] Sharma V. K.., Hansch, R., Mendel R.R. and Schulze J. (2004). A highly efficient plant regeneration system through multiple shoot differentiation from commercial cultivars of barley (*Hordeum vulgare* L.) using meristematic shoot segments excised from germinated mature embryos. Plant Cell Rep. 23: 9-16.
[43] Sharma V.K., Hansch R., Mendel R.R. and Schulze J. (2005). Mature embryo axis-based high frequency somatic embryogenesis and plant regeneration from multiple cultivars of barley (*Hordeum vulgare*). J.Exp. Bot. 56: 1913-1922.
[44] Svetla D.Y., Sara G., Ervin F., Simcha L.Y. and Moshe A.F. (2003). Auxin type and timing of application determine the activation of the developmental program during in vitro organogenesis in apple. Plant Sci. 165: 299-309.
[45] Thomas J.C. and Katterman F.R. (1986). Cytokinin activity induced by thidiazuron. Plant Physiol. 81: 681-683.

[46] Tian W., Rance I., Sivamani E., Fauquet C. and Beachy R. N. (1994). Improvement of plant regeneration frequency *in vitro* in indica rice. Chin. J. Genet. 21:1–9; 1994.
[47] Varshney A., Jain S. and Kothari S.L. (1999). Plant regeneration from mature embryos of 20 cultivars of wheat. Cerel Res. Comm.27 (1-2): 163- 170.
[48] Vasil I.K.. (2007). Molecular genetic improvement of cereals: transgenic wheat (*Triticum aestivum* L.). Plant Cell Rep. DOI 10.1007/s00299-007-0338-3.
[49] Vasil V., Srivastava V., Castillo A.M. and Vasil I.K. (1993). Rapid production of transgenic wheat plants by direct bombardment of cultured immature embryos. Bio/Technology, 11:1553–1558.
[50] Vikrant and Rashid A. (2002). Induction of multiple shoots by thidiazuron from caryopsis cultures of minor millet (*Paspalum scrobiculatum* L.) and its effect on the regeneration of embryogenic callus cultures. Plant Cell Rep. 21:9–13.
[51] Vikrant, Maragathamani R. and Khurana P. (2012). Somatic embryogenesis from mature caryopsis culture under abiotic stress and optimization of Agrobacterium-mediated transient GUS gene expression in embryogenic callus of rice (*Oryza sativa* L.), Journal of Phytology. 4(5):16-25.
[52] Weeks, J.T., Anderson, A.D. and Blechl, A.E. (1993). Rapid production of multiple independent lines of fertile transgenic wheat (*Triticum aestivum*). Plant Phys. 1077-1084.
[53] Yu Y., Wang J., Zhu M.L. and Wei Z.M. (2008). Optimization of mature embryo-based high frequency callus induction and plant regeneration from elite wheat cultivars grown in China. Plant Breed. 127:249–255.
[54] Zale M. J., Borchardt-Wier H., Kidwell K.K. and Steber C.M. (2004). Callus induction and plant regeneration from mature embryos of a diverse set of wheat genotypes. Plant Cell Tissue Organ Cult. 76: 227-281.