*Pak. J. Bot.*, 42(1): 639-652, 2010.

# EFFECT OF CULTIVARS AND CULTURE MEDIUM ON CALLUS FORMATION AND PLANT REGENERATION FROM MATURE EMBRYOS OF WHEAT (*TRITICUM AESTIVUM* L.)

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**Abstract**

Experiments were conducted to investigate callus induction, plant regeneration and somaclonal variations in 9 locally developed wheat cultivars viz., Ghaznavi-98, Fakhr-e-Sarhad, Inqilab-91, Tatara, Takbeer, Margalla, Pirsabak-85, SARC-3 and Khattakwal and one line from ICARDA (ICP-3) on hormones free and various concentrations of 2,4-dichorophenoxyaceitc acid (2, 4-D) in LS/MS medium. Cultivars responded differently to medium and 2, 4-D concentration for callus induction. Maximum calli induction (67.5%) was noted in ICP-3 followed by SARC-3 (65.5%) on MS. In our studies MS medium was more effective for all the wheat cultivars compared with LS medium for callus induction. When the effect of different concentration of 2, 4-D on callus induction was studied, MS medium containing 2 mg $l^{-1}$ 2, 4-D produced the greatest number of calli. Morphology of these cultivars were further studied on both LS/MS medium containing only 2 mg $l^{-1}$ 2, 4-D. Embroyogenic and non-embryogenic calli were observed in all the cultivars studied. Embryogenic calli were generally compact and rapidly growing whereas non-embryogenic calli were loose and slow growing. For regeneration, LS/MS medium was supplemented with various combinations of IAA and BAP. Significant differences were detected in plant regeneration, culture efficiency and regeneration capacity when mature embryos of 9 locally developed cultivars and one line from ICARDA (ICP-3) were compared. Six cultivars responded efficiently to LS medium while four showed better performance on MS medium for plant regeneration. Majority of the somaclones regenerated in this study were found to be inferior for agronomic traits except plant height, days to heading and maturity when compared with their respective controls. Our results showed that callus derived from this wheat cultivar is amendable and could be used for genetic transformation studies.

## Introduction

Calli formation and plant regeneration from dicotyledonous species have been extensively reported. However, the process for monocotyledonous species has been less successful. Different explants sources have been used for the induction of callus and regenerable wheat culture: shoot tips (Viertel & Hess, 1996), leaf blade (Yan & Zhao, 1982), root (Abe & Futsugara, 1985), inflorescences (Ozias-Akins & Vasil, 1982), anthers (Last & Brettel, 1990; Zhou *et al*., 1991), isolated microspores (Mejza *et al*., 1993) and immature embryos (Ozias-Akins & Vasil, 1982, 1983; Eapen & Rao, 1982; Koetije *et al*., 1989; Vasil *et al*., 1990; Li *et al*., 1992 a and b; He *et al*., 1992) and mature seed (Masuda *et al*., 1989). Callus induction and regeneration potential are affected by cultivars, explants and carbohydrates sources, plant growth regulators, basal salts of culture medium and culture conditions. Cultivars and culture medium play an important role in callus induction and plant regeneration in wheat (Ozias-Akins & Vasil, 1983; He

*et al*., 1986; Armstrong *et al*., 1987; Ben Amer *et al*., 1992; Machii *et al*., 1998), barley (Luhrs and Lorz, 1987; Komatsuda *et al*., 1989), triticale (Sharma *et al*., 1981) and rye (Linacero & Vasquez, 1986, 1990; Rakocy-Trojanowska and Malepszy, 1993, 1995). Several studies have been reported on the effect of 2, 4-D on callus induction and growth from explants of graminious species (Conger *et al*., 1978, 1982; Deambrogio & Dale, 1980; Lu *et al*., 1982; Thomas & Scott, 1985; Fladung & Hesselbach, 1986). In most of these studies, an optimal concentration of 2, 4-D for callus induction and growth was investigated which varied with the species. The use of mature embryo has many advantages over immature tissues as starting material for *In vitro* callus induction and regeneration (i) the embryogenic calli induced from mature seeds can be effectively used for genetic transformation (Li *et al*., 1990; Hiei *et al*., 1994). (ii). unlike the immature embryo, the physiological state of the mature embryo shows minimal variability. (iii). the need to grow donor plant material in green house can be avoided thus reducing the cost and (iv)-it also reduces the physiological variation of *In vitro* regeneration response of explants derived from immature tissues (Vasil *et al*., 1993; Becker *et al*., 1994; Maes *et al*., 1996). Recent development in plant genetic engineering have opened several opportunities for the development of artificially improved cultivars of cereals particularly wheat using transformation technology in which different genes of interest can be introduced into the existing cultivar. However, due to the lack of an effective callus induction and regeneration system, the process is inefficient and costly. The aim of this project was to study the effect of different wheat cultivars, culture medium and hormones on callus induction, plant regeneration and somaclonal variations.

## Materials and Methods

**Plant materials:** Mature seeds of nine locally developed wheat (*Triticum aestivum* L.) cultivar viz., Ghaznavi-98, Fakhr-e-Sarhad, Inqilab-91, Tatara, Takbeer, Margalla, Pirsabak-85, SARC-3 and Khattakwal and one line from ICARDA (ICP-3) were used for embryogenic callus induction and plant regeneration. The pedigrees of these cultivars are given in Table 1. For Somaclonal variation only six cultivars were studied (Ghaznavi-98, Fakhr-e-Sarhad, Tatara, SARC-3, Khattakwal and ICP-3). Mature dehusked seeds were first soaked in sterilized distilled water for 24 hours. The soaked seed were then sterilized sequentially with 70% ethanol for 15 minutes and 25% (v/v) commercial bleach (Sodium hypochlorite; NaOCl) supplemented with 1-2 drops of Tween-20 (Sigma) for 20 minutes with constant shaking and were then rinsed several times thoroughly with sterilized water. Embryos were excised from the sterilized seeds and were used for callus induction and plant regeneration.

**Callus induction:** Two basal medium, LS (Linsmaier & Skoog, 1965) and MS (Murashige & Skoog, 1962) were tested for the induction of embryogenic calli. Both the medium mentioned above were supplemented with 3% sucrose and 0.8% agar. Each medium was divided into five groups for the addition of different concentration of different concentrations of 2, 4-D (0, 1, 2, 3 and 4 mg $l^{-1}$). Prior to autoclaving, pH was adjusted to 5.8. After autoclaving, medium was poured in Petri dishes. Ten embryos were cultured on each Petri dish for each treatment of the different cultivars. The experiment was replicated ten times. Cultures were incubated in growth chamber at 25 ± 2C in the dark (80% RH). After 10 days, cultures were monitored daily for callus formation by which time the embryo had turned into undifferentiated mass. Callus frequency was calculated in percentage whereas its color and type was recorded visually. Calli were cultured on fresh medium with an interval of 30 days for 3-4 cycles.

**Table 1. Wheat cultivars and their pedigree.**

| S. No. | Cultivar/Line | Pedigree |
|---|---|---|
| 1. | Ghanzavi | 98 JUP/Bjyy//Ures |
| 2. | Fakhr-e-Sarhad | PFAU S/SERI/BOWS |
| 3. | Inqilab-91 | WL-711/CROWS |
| 4. | Tatara | JUP/ALDS??KLTS/3VEES |
| 5. | Takbeer | ATTILA |
| 6. | Margalla-98 | MAYO54E/LR64/1 TACS |
| 7. | Pirsabak-85 | KVZ/BUSHS/KAL/BB |
| 8. | ICP-3 | Line form ICARDA |
| 9. | SARC-3 | Blue Silver/Khushal69 |
| 10. | Khattakwal | Land race of Southern Part of NWFP, Pak |

**Table 2. Combination of IAA and BAP for plant regeneration in LS or MS medium.**

| Medium | IAA + BAP combination |
|---|---|
| RM-1 | 0.1 mg $l^{-1}$ IAA + 0.5 mg $l^{-1}$ BAP |
| RM-2 | 0.1 mg $l^{-1}$ IAA + 1.0 mg $l^{-1}$ BAP |
| RM-3 | 0.2 mg $l^{-1}$ IAA + 0.5 mg $l^{-1}$ BAP |
| RM-4 | 0.2 mg $l^{-1}$ IAA + 1.0 mg $l^{-1}$ BAP |

**Plant regeneration:** The embryogenic calli were transferred onto regeneration medium (LS/MS) which was the same as induction medium with the exception of the inclusion of various concentrations of IAA and BAP instead of 2, 4-D (Table 2). Plants were regenerated at 25 ± 2 C, 16/8 hours day night photoperiod (80% RH). When plants were 12 cm height, those with healthy roots systems were transferred into soil and grown in glass house.

**Somaclonal variation:** Seeds from 6 cultivars (Ghaznavi, Fakhre-Sarhad, Tatara, SARC-3, ICP-3 and Khattakwal) of the regenerated plants were collected and were further studied for somaclonal variations.

## Results

Callus induction was significantly affected by cultivar choice. When these cultivars were cultured on LS medium, Ghaznavi-98 produced the greatest number of calli (62.5%) while Tatara performed poorly when compared with other cultivars cultured on LS medium (Fig. 1A). The highest response was noticed in ICP-3 (67.5%) while callus induction was lowest (52%) in Margalla when cultured on MS medium and compared with other cultivars cultured on the same medium (Fig. 1 B). Analysis of the data also indicated that callus induction was significantly ($p < 0.05$) affected by media. Our result suggested that maximum (61.9%) calli were induced when MS medium was used compared with LS medium (55.25%) (Fig. 1A vs. 1B). When the effect of different concentrations of 2, 4-D was taken into account (Fig. 1C), it was revealed that various concentrations of 2, 4-D had a significant ($p<0.05$) effect on callus induction. Calli regeneration was more successful on MS media while optimum concentration of 2, 4-D for both media was 2 mg $l^{-1}$ (Fig. 1A, B and C).

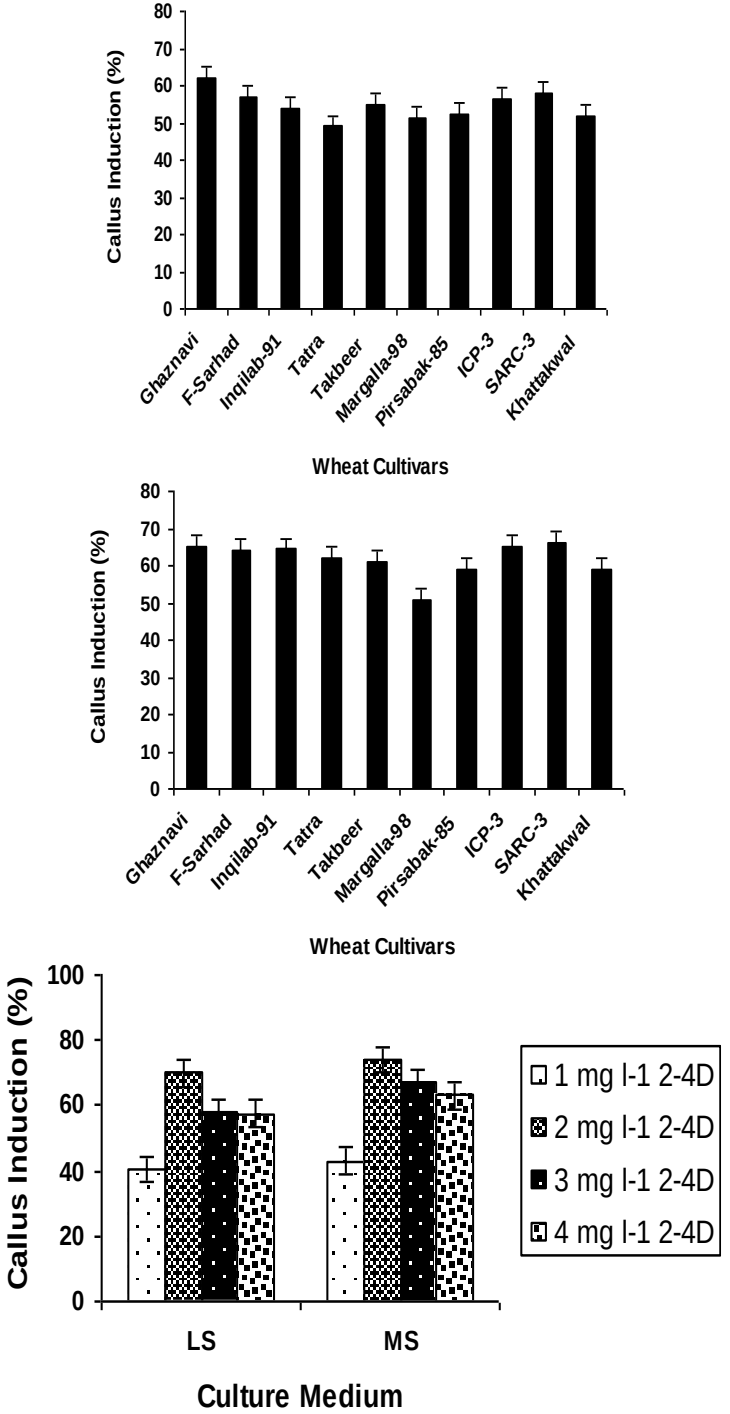


Fig. 1. Response of different wheat cultivars to (A) LS (B) MS medium and (C) different concentrations of 2,4-D for callus induction (Bar shows ±1LSD at $p<0.05$).

**Table 3. Callus morphology of different wheat cultivars on LS or MS medium containing 2 mg $l^{-1}$ 2, 4-D.**

| Cultivars | LS medium | | | MS medium | | |
|---|---|---|---|---|---|---|
| | Quality | Color | Type | Quality | Color | Type |
| Ghaznavi-98 | ++ | C.green | Compact | ++ | C.green | Compact |
| F-Sarhad | ++ | Yellow | Hard | +++ | White | Hard |
| Inqilab-91 | + | C.green | Compact | + | C.green | Compact |
| Tatara | + | Yellow | Soft | ++ | Yellow | Soft |
| Takbeer | + | Creamy | Friable | + | Creamy | Friable |
| Margalla | + | C.green | Compact | + | C.green | Compact |
| Pirsabak-85 | + | Brown | Friable | + | Brown | Friable |
| ICP-3 | ++ | C.green | Compact | +++ | C.green | Compact |
| SARC-3 | ++ | C.green | Compact | +++ | C.green | Compact |
| Khattakwal | ++ | Yellow | Soft | ++ | Yellow | Soft |

C.green = Creamy green, + = Poor, ++ = Average, +++ = Best

Analysis of the data suggested that various cultivars, medium type and different concentrations of 2, 4-D had a significant ($p<0.05$) effect on percent embryogenic calli. Cultivar Fakhr-e-Sarhad produced maximum embryogenic calli (69.82% or 63.17%) when cultured on MS or LS medium (Fig. 2 A and B). Again, MS medium supplemented with 2 mg $l^{-1}$ 2, 4-D resulted in maximum (75.8%) embryogenic calli when compared with LS medium (67.9%) (Fig. 2C). After establishing that 2 mg $l^{-1}$, 2, 4-D was effective for the induction of maximum calli compared with other concentration, the morphology of these calli were studied by sub-culturing of calli on LS or MS medium containing 2 mg $l^{-1}$ 2, 4-D. LS medium containing 2 mg $l^{-1}$ 2, 4-D induced more yellow white calli when compared with the color of calli induced on MS medium containing the same concentrations of 2, 4-D. Similarly, morphology of the various cultivars responded differently to LS or MS medium containing 2 mg $l^{-1}$ 2, 4-D. Calli of three cultivars (Fakhr-e-Sarhad, ICP-3 and SARC-3) induced on MS medium supplemented with 2 mg $l^{-1}$ 2, 4-D were best when compared with the calli induced on LS medium containing the same amount of 2, 4-D (2 mg $l^{-1}$). Cultivars Takbeer, Margalla and Pirsabak-85 induced poor quality of calli on both the medium (LS/MS) containing 2 mg $l^{-1}$ 2, 4-D (Table 3; Fig. 3A vs C). Analysis of the data revealed that all the cultivars showed variable response to different medium for regeneration. In all cultivars except Khattakwal, plant regeneration was fast and similar. Our data suggested that significant ($p<0.05$) highest mean regeneration efficiency was recorded by Fakhr-e-Sarhad (27.5% or 21.75%) whereas Khattakwal noted lowest mean regeneration efficiency (7.75% or 7.50%) when regenerated on LS or MS medium (Fig. 4 A and B; Fig. 5 A and B). Our results indicated that for regeneration, LS medium was superior to MS medium respectively (Fig 4 A and. B). When the effect of different hormones combinations on plant regeneration was considered we found that both medium (LS/MS) containing 0.1 mg $l^{-1}$ IAA and 0.5 mg $l^{-1}$ BAP (RM-1) regenerated the maximum plants (20% and 25.5% respectively) when compared with other combinations. Whereas minimum plants were regenerated when LS/MS medium was supplemented with 0.2 mg $l^{-1}$ IAA and 1.0 mg $l^{-1}$ BAP (Fig. 4C).

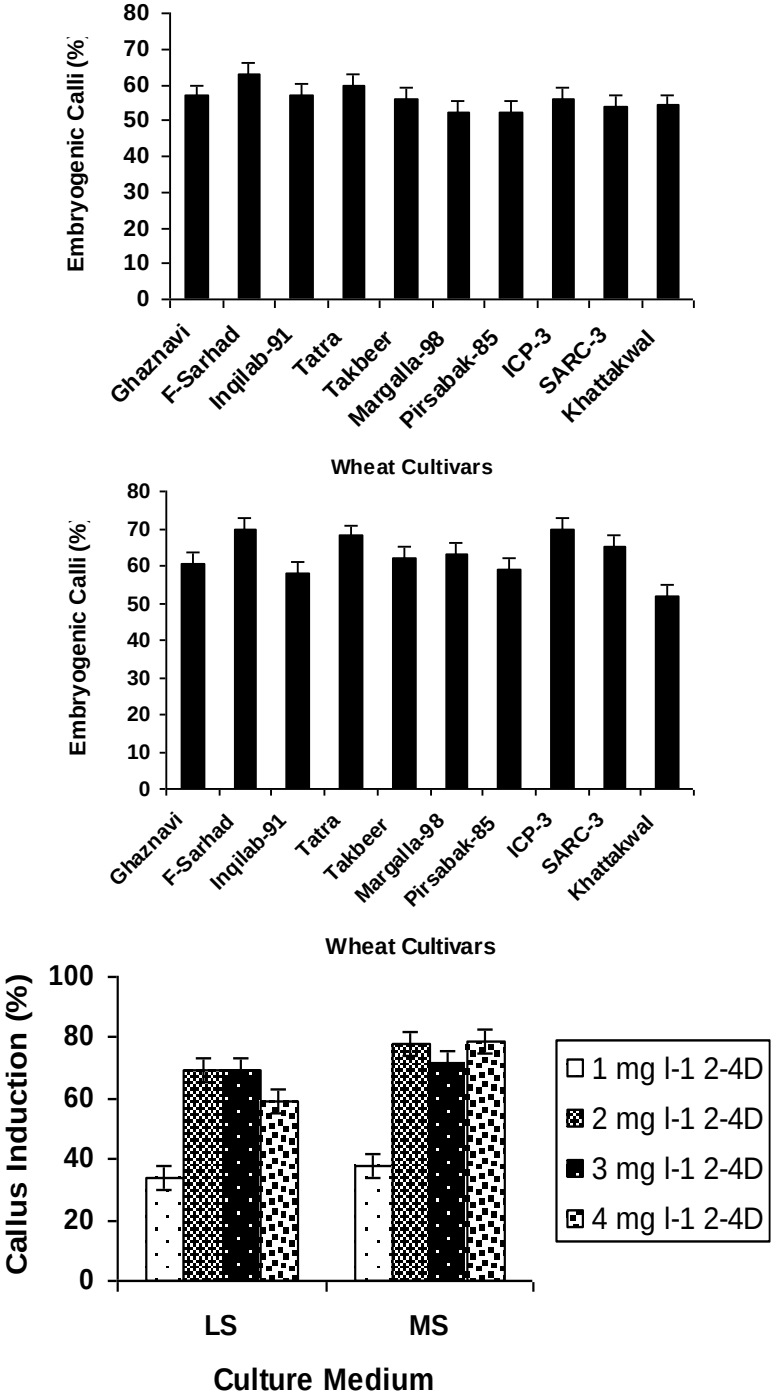


Fig. 2. Induction of embryogenic calli by different wheat cultivars on (A) LS (B) MS medium containing different concentrations of 2, 4-D (C) (Bar shows ±1LSD at $p < 0.05$).

**Table 4. Performance of somaclones derived from different wheat cultivars with their respective controls.**

| Character | Source | Ghaz | F-Sarhad | Tatara | SARC-3 | ICP-3 | K/wal |
|---|---|---|---|---|---|---|---|
| Ear | S/clone | 111 | 94 | 105 | 88 | 101 | 121 |
| Emergence (day) | Control | 102 | 107 | 98 | 85 | 91 | 116 |
| % Increase/Decrease over | Control | 8.8 | 13.03 | 7.14 | 3.53 | 10.98 | 4.31 |
| Maturity (day) | S/clone | 163 | 157 | 165 | 162 | 166 | 159 |
| | Control | 158 | 164 | 160 | 162 | 158 | 153 |
| % Increase/Decrease over | Control | 3.16 | 4.46 | 3.13 | | 5.06 | 3.92 |
| Plant | S/clone | 97.5 | 105.3 | 102.1 | 101.3 | 113.1 | 101.7 |
| Height (cm) | Control | 102.3 | 116.6 | 121.0 | 119.1 | 102.1 | 119.5 |
| % Increase/Decrease over | Control | 4.9 | 10.73 | 18.51 | 17.57 | 10.77 | 17.50 |
| Stem | S/clone | 4.6 | 4.1 | 4.3 | 3.9 | 3.5 | 3.6 |
| Diameter (cm) | Control | 4.3 | 4.5 | 3.9 | 4.3 | 3.7 | 4.1 |
| % Increase/Decrease over | Control | 6.98 | 9.76 | 10.25 | 10.26 | 5.71 | 3.88 |
| Flag | S/clone | 43.9 | 47.5 | 36.2 | 41.3 | 33.2 | 32.5 |
| Leaf area $(cm)^3$ | Control | 51.2 | 57.3 | 47.2 | 49.5 | 44.2 | 47.3 |
| % Increase/Decrease over | Control | 16.63 | 20.63 | 30.39 | 19.85 | 33.13 | 45.54 |
| Spike | S/clone | 11.7 | 11.1 | 9.7 | 13.1 | 9.0 | 7.2 |
| Length (cm) | Control | 13.5 | 11.4 | 10.0 | 13.0 | 11.7 | 9.1 |
| % Increase/Decrease over | Control | 15.38 | 2.70 | 3.09 | 0.77 | 30.0 | 26.39 |
| Grains | S/clone | 18 | 20 | 18 | 20 | 18 | 13 |
| Per spike | Control | 22 | 24 | 18 | 21 | 19 | 16 |
| % Increase/Decrease over | Control | 22.22 | 20.0 | | 5.0 | 5.55 | 23.0 |

Ghaz = Ghaznavi-98, F/Sarhad = Fakhr-e-Sarhad, K/wal = Khattakwal

Somaclones generally did not differ physiologically from their respective control except for days from sowing until heading and maturity with the somaclones. The somaclones responded differently for heading and maturity and ranged from 121 days for heading and 157-166 days for maturity. Mean values of the somaclones regenerated from Fakhr-e-Sarhad produced heads 13 days earlier which resulted in early maturity for the same cultivar by 7 days than their parents. While other somaclones induced from different cultivars produced heads 2-10 days later and thus matured later by 8 days than their respective control plants (Table 4). Taller plants (11 cm) were recorded in somaclones of Pirsabak-85 whereas the somaclones derived from the other cultivars were shorter (4.8 to 17.8 cm) when compared with their respective controls. Thicker stem diameter was recorded in somaclones of Ghaznavi-98 and Tatara while somaclones from the other cultivars has thinner stem (0.3-0.5 cm). Decreased flag leaf area was also observed in all the somaclones which ranged from 7.3 to 14.8 cm. Spike length was also decreased in all the somaclones except the somaclone derived from Takbeer (Table 4).

## Discussion

All the cultivars of wheat analyzed provided good levels of callus induction but differences were observed between cultivars, type of medium and concentration of 2, 4-D with respect to their percentage of calli formed and their morphology. Statistical analysis of our results suggested that frequencies of callus induction varied from 65% to 49.75% depending on the type of medium and cultivar. The highest percentage of calli was reported by cultivar ICP-3 when cultured on MS medium while lowest by Tatara. It has been reported that callus induction frequency ranges from 44% to 89% in six cultivars of

*T. durum*, and 90-100% calli with 4 durum wheat cultivars (He *et al*., 1988; Bommineni & Jauher, 1996). Callus induced on MS medium produced maximum calli when compared with LS medium. No callus was induced from any cultivars under study when 2, 4-D, free medium was used. Similar results are also reported by Joseph *et al*., (2000). Seventy five percent of the calli were observed in MS medium containing 2 mg $l^{-1}$ 2, 4-D when compared with LS and other 2, 4-D concentrations (Joseph *et al*., 2000). Our results also confirm those reported by Zheng & Konzak (1999), Bronsema *et al*., (2001) and Lee *et al*., (2002). In general, 2, 4-D is the most commonly used auxin to induce somatic embryogenesis (Ozias-Akins & Vasil, 1982, 1983; Lu *et al*., 1984; MacKinnon *et al*., 1987; Elena and Ginzo, 1988; Duditis *et al*., 1991; Li *et al*., 1992 a and b; Racz *et al*., 1993; Joseph *et al*., 1999; Lee *et al*., 2002). However, the concentration of 2, 4-D may vary with different plant species. After establishing the role of 2, 4-D at 2 mg $l^{-1}$, morphology of the different cultivars under study was investigated by sub-culturing the callus on LS/MS medium containing the same concentration of 2, 4-D (2 mg $l^{-1}$). Our results suggested that the generation of embryogenic calli was more efficient on MS medium containing 2 mg $l^{-1}$ of 2, 4-D when compared with the other concentrations of 2, 4-D on MS or LS medium. More yellow calli were induced by LS medium when compared with MS medium containing the same amount of 2, 4-D (2 mg $l^{-1}$). LS or MS medium showed similar levels of compact callus production. The cultivars under investigation responded differently with calli varying in color from white to yellow and texture varying from compact to loose. Calli of three cultivars (Fakhr-e-Sarhad, ICP-3, SARC-3) induced on MS were best compared with the other cultivars cultured on MS or LS medium. Belarmino *et al*., (1992) reported different colored and textured calli in *Iopmoea batatas* L.

Our result indicated that all the cultivars showed variable response to the type of medium and their hormone composition for regeneration. Our results also shows that the highest plant regeneration was obtained when cultivar Fakhre-e-Sarhad was regenerated on LS medium compared with other cultivars regenerated on LS or MS medium. The data also suggested that plant regeneration was more when LS medium was used instead of MS medium. Analysis of the data concerning the effect of different hormone combinations revealed that greatest plant regeneration was obtained in both the medium (LS/MS) containing 0.1 mg $l^{-1}$ IAA and 1.0 mg $l^{-1}$ BAP. The somaclones derived from these cultivars were not significantly different from their controls except for plant height, days to heading and maturity. Somaclones derived from five cultivar (Fakhr-e-Sarhad, Tatara, SARC-3, ICP-3 and Khattakwal) were short stature and can be useful for the development of shorter cultivars for lodging resistance. Similarly, Fakhr-e-Sarhad headed earlier which resulted in their early maturity. This early maturity character can be exploited for the development of early maturing cultivar for dry regions to avoid water stress at the latter stages of maturity.

## Conclusion

The various cultivars used in this study responded differently to medium type for callus induction and plant regeneration. MS medium containing 2 mg $l^{-1}$ 2, 4-D was found superior for the induction of callus and embryogenic calli when compared with LS medium. However, for plant regeneration LS medium containing 0.1 mg $l^{-1}$ IAA and 1.0 mg $l^{-1}$ BAP was suitable instead of MS medium. Somaclones derived from these cultivars were useful only for the development of shorter and early maturing cultivars.

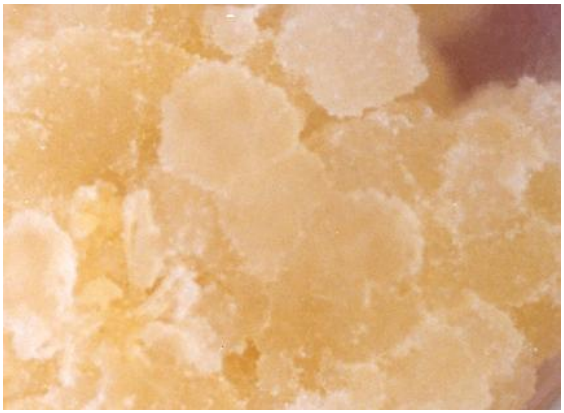

Fig. 3A (Best quality)

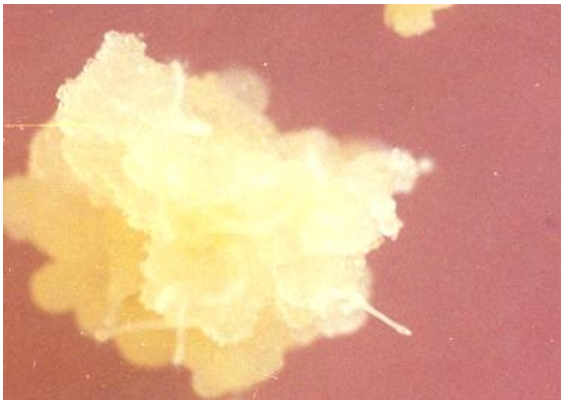

Fig. 3B (Average quality)

Fig. 3C (Poor quality)

Fig. 3. Photograph of different classes of calli (A). Best quality (B) Average quality and (C) Poor quality.

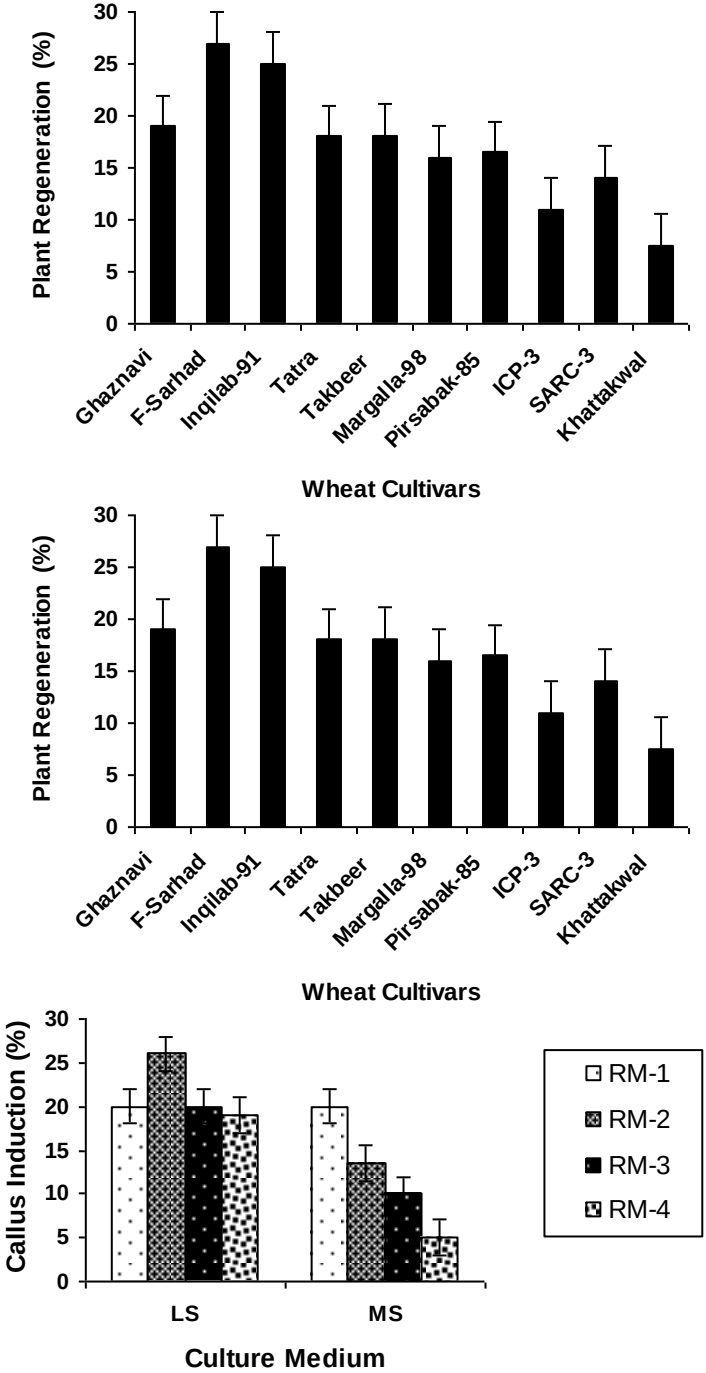


Fig. 4. Plant regeneration of different wheat cultivars on (A) LS (B) MS medium containing different concentration of IAA and BAP (C) on plant regeneration (Bar shows ±1LSD at $p<0.05$).

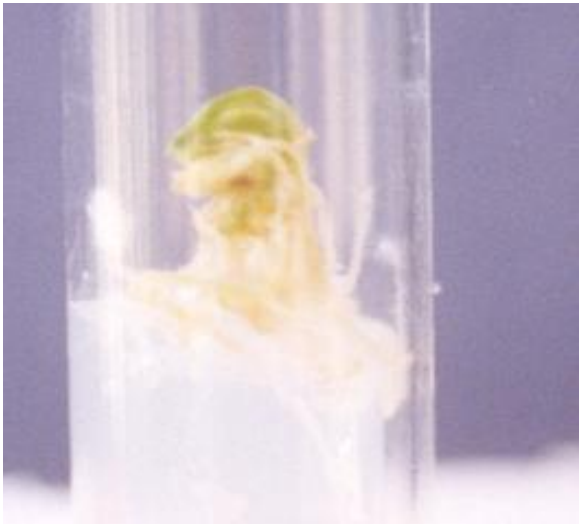

Fig. 5A (Regeneration efficiency of Fakhre-Sarhad)

Fig. 5 B (Regeneration efficiency of KhattakWal)

Fig. 5. Plant regeneration of (A) Fakhre-Sarhad and (B) Khattakwal when regenerated on LS or MS medium.

**References**

Abe, T. and Y. Futsugara. 1985. Efficient plant regeneration by somatic embryogenesis from root callus tissues of rice. *J. Plant Physiol.*, 121: 111-118.

Armstrong, T.A, S.G. Metz and P.N.N. Mascia. 1987. Two regeneration system for the production of haploid plants from wheat anther culture. *Plant Sci.*, 5: 231-237.

Becker, D., R. Brettschneider and H. Lorz. 1994. Fertile transgenic wheat from microprojectile bombardment of scuteller tissue. *Plant J.*, 5: 299-307.

Belarmino, M.M., T. Abe and T. Sashara. 1992. Efficient plant regeneration form leaf calli of *Ipomoea batatas* L. and its related species. *Jap. J. Breed.*, 42: 109-114.

Ben Amer, I.M., A.J. Worland and A. Borner. 1992. *In vitro* culture variation of wheat caused by genes affecting plant growth habit *in vivo. Euphytica,* 61: 233-240.

Bommineni, V.R. and P.P. Jauher. 1996. Regeneration of plantlets through isolated scutellum culture of durum wheat. *Plant Sci.,* 116 197-207.

Bronsema, F.B.F., W.J.F. van Oostveen and A.A.M. van Lammeren. 2001. Influence of 2,4-D, TIBA and 3,5-D on the growth response of maize embryo. *Plant Cell, Tiss. and Org. Cult.,* 65: 45-56.

Conger, B.V., J.V. Carabia and K.W. Lowe. 1978. Comparison of 2, 4-D and 2,4 5-T on callus induction and growth in three gramineae species. *Environ. Exp. Bot.,* 18: 163-168.

Conger, B.V., L.L. Hilenski, K.W. Lowe and J.V. Carabia. 1982. Influence of different auxins at varying concentration on callus induction and growth from embryo and leaf tip explants in gramineae. *Environ. Exp. Bot.,* 22: 39-48.

Deambrogio, E. and P.J. Dale. 1980. Effect of 2, 4-D on the frequency of regenerated plants in barley and on genetic variability between them. *Cereal Res. Commun.,* 8: 417-422.

Duditis, D., L. Bogre and J.C. Gyorgyey. 1991. Molecular and cellular approaches to the analysis of plant embryo development from somatic cells *In vitro.* J. *Cell Sci.,* 99: 475-484.

Eapen, S. and P.S. Rao. 1982. Callus induction and plant regeneration from immature embryos of rye and *Triticale*. *Plant Cell, Tiss. and Org. Cult.,* 1: 221-227.

Elena, E.B. and H.D. Ginzo. 1988. Effect of auxin levels on shoot formation with different embryo tissues from a cultivar of a commercial hybrid of wheat (*Triticum aestivum* L.). *Plant Physiol.,* 132: 600-603.

Fladung, M. and J. Hesselbach. 1986. Callus induction and plant regeneration in *Panicum bisulcatum* and *Pinicum milioides. Plant Cell Rep.,* 3: 169-173.

He, D.G., G. Tanner and K.J. Scott. 1986. Somatic embryogenesis and morphogenesis in callus derived from the epiblast of immature embryo of wheat (*Triticum aestivum* L.). *Plant Sci.,* 45: 119-124.

He, D.G., Y.M. Yang and K.J. Scott. 1988. A comparison of scutellum callus and epiblast callus induction in wheat: the effect of cultivar, embryo age and medium. *Plant Sci.,* 57: 225-233.

He, D.G., Y.M. Yang and K.J. Scott. 1992. Plant Regeneration from protoplast of wheat (*Triticum aestivum* L. cv Hartog). *Plant Cell Rep.,* 11: 16-19.

Heyser, J.W., M.W. Nabors, C. Mackinnon, T.A. Dykes, K.J. Demott, D.C. Kautzman and Z. Mujeeb-Kazi. 1985. Long term, high frequency plant regeneration and the induction of somatic embryogenesis in callus culture of wheat (*Triticum aestivum* L.). *Pflanzenzuchtg,* 94: 218-233.

Hiei, Y., Y. Ohta, T. Komari and T. Kumashiro. 1994. Efficient transformation of rice (*Oryza sativa* L.) mediated by *Agrobacterium* and sequence analysis of the T-DNA. *Plant J.,* 6: 271-282.

Joseph, T., H.H. Yeoh and C.S. Loh. 1999. Cyanogenesis in somatic embryos and plantlets of cassava (*Manihot esculenta* Crantz). *J. Sci. Food Agric.,* 79: 1071-1074.

Joseph, T., H.H. Yeoh and C.S. Loh. 2000. Somatic embryogenesis, plant regeneration and cyanogenesis in *Manihot glaziovii* Muell. Arg. (*Ceara rubber*). *Plant Cell Rep.,* 19: 535-538.

Koetije, D.S., H.D. Grimes, Y.C. Wang and T.K. Hodes. 1989. Regeneration of indica rice (*Oryza sativa* L.) from primary callus derived from immature embryos. *J. Plant Physiol.,* 134: 184-190.

Komatsuda, T.S., S. Enomoto and K. Nakjima. 1989. Genetics of callus proliferation and shoot differentiation in barley. *J. Hered.,* 80: 345-350.

Last, D.I. and R.I.S. Brettel. 1990. Embryo yield in wheat anther culture is influenced by the choice of sugar in the culture medium. *Plant Cell Rep.,* 9: 14-16.

Lazar, M.D., G.B. Collins and W.E. Vian. 1983. Genetic and environmental effects on the growth and differentiation of wheat somatic cell cultures. *J. Hered.,* 74: 353-357.

Lee, K., H. Jeon and M. Kim. 2002. Optimization of a mature embryo-based *In vitro* culture system for high-frequency somatic embryogenic calli induction and plant regeneration from *japonica* rice cultivars. *Plant Cell, Tiss. and Org. Cult.,* 71: 244-2002.

Li, Z., M.D. Burow and N. Murai. 1990. High frequency generation of fertile transgenic rice plants after PEG-mediated protoplast transformation. *Plant Mol. Biol. Rep.,* 8: 276-291.

Li, Z.Y., G.M. Xia and H.M. Chen. 1992 a. Somatic embryogenesis and plant regeneration from protoplast isolated from embryogenic cell suspension of wheat (*Triticum aestivum* L.). *Plant Cell, Tiss. and Org. Cult.,* 28: 79-82.

Li, Z.Y., G.M. Xia, H.M. Chen and G.Q. Guo. 1992 b. Plant regeneration from protoplast derived from embryogenic cell suspension of wheat (*Triticum aestivum* L.). *J. Plant Physiol.,* 139: 714-718.

Linacero, R. and A.M. Vasquez. 1986. Somatic embryogenesis and plant regeneration from leaf tissues of rye (*Sacale cereals* L.). *Plant Sci.,* 44: 219-222.

Linacero, R. and A.M. Vasquez. 1990. Somatic embryogenesis from immature inflorescences of rye. *Plant Sci.,* 72: 253-258.

Linsmaier, E. and F. Skoog. 1965 Organic growth factor requirements of tobacco tissue cultures. *Physiol. Plant.,* 18: 100-127.

Lu, C.Y, I.K. Vasil and P. Ozias-Akins. 1982. Somatic embryogenesis in *Zea mays* L. *Theore. Appl. Gene.,* 62: 109-112.

Lu, C.Y., S.F. Chandler and I.K. Vasil. 1984. Somatic embryogenesis and plant regeneration from cultured immature embryos of rye (*Scale cereale* L.). *Plant Physiol.,* 115: 237-244.

Luhrs, R. and H. Lorz. 1987. Plant regeneration in vitro from embryogenic cultures of spring and winter type barley (*Hordium vulgare* L.) cultivar. *Theore. Appl. Gene.,* 75: 16-25.

Mace-Kinnon, C., G. Gunderson and M.W. Nabors. 1987. High frequency plant regeneration by somatic embryogenesis from callus of mature explants of bread wheat (*Triticum aestivum* L.) and grain sorghum (*Sorghum bicolor*). *In vitro Cell Devel. Biol*., 23: 443-448.

Machii, H., H. Mizuno, T. Hirabayashi, H. Li and T. Hagio. 1998. Screening wheat cultivars for high callus induction and regeneration capability from anther and immature embryo cultures. *Plant Cell, Tiss. and Org. Cult.,* 53: 67-74.

Maes, O., R.N. Chibbar, K. Caswell, N. Leung and K.K. Kartha. 1996. Somatic embryogenesis from isolated scutella of wheat: effect of physical, physiological and genetic factors. *Plant Sci.,* 121: 75-84.

Masuda, K., A. Kudo-Shirator and M. Inoue. 1989. Callus transformation and plant regeneration from rice protoplasts purified by density gradient centrifugation. *Plant Sci.,* 62: 237-246.

Mejza, S.J., V. Morgant, D.E. Di Bona and J.R. Wong. 1993. Plant regeneration from isolated microspores of *Triticum aestivum. Plant Cell Rep.,* 12: 149-153.

Mohmand, A.S. and M. W. Nabors. 1990. Somaclonal variant plants of wheat derived from mature embryo explants of three cultivars. *Plant Cell Rep.,* 8: 558-560.

Murashige, T. and F. Skoog. 1962. A revised medium for rapid growth and bioassay with tobacco culture. *Physiol. Plant.,* 15: 73-97.

Ozias-Akins, P. and I.K. Vasil. 1982. Plant regeneration from cultured immature embryos and inflorescences of *Triticum aestivum* L.: evidence for somatic embryogenesis. *Protopl.,* 11: 95-105.

Ozias-Akins, P. and I.K. Vasil. 1983. Improved efficiency and normalization of somatic embryogenesis in *Triticum aestivum* L. *Protopl.,* 117: 40-44.

Racz, I., E. Paldi, D. Lasztity, B. Buzas and M. Aczel. 1993. Callus cultures and plant regeneration from mature embryos in winter wheat. *Acta Agron Hun.,* 42: 255-260.

Rakocy-Trojanowska, M. and S. Malepszy. 1993. Genetic factors influencing the regeneration ability of rye (S*acale cereale* L.) I Immature inflorescence. *Theore. Appl. Gene.,* 86: 406-410.

Rakocy-Trojanowska, M. and S. Malepszy. 1995. Genetic factors influencing the regeneration ability of rye (S*acale cereale* L.) II Immature embryo. *Euphytica,* 83: 233-239.

Rueb, S., M. Leneman, R.A. Schilperoort and L.A.M. Hensgens. 1994. Efficient plant regeneration through somatic embryogenesis from callus induced on mature rice embryos (*Oryza sativa* L.). *Plant Cell, Tiss. and Org. Cult.,* 36: 259-264.

Sharma, G.C., L.L. Bello, V.T. Sapra and C.M. Paterson. 1981. Callus initiation and plant regeneration from *Triticale* embryos. *Crop Sci.,* 21: 113-118.

Thomas, M.R. and K.J. Scott. 1985. Plant regeneration by somatic embryogenesis from callus initiated from immature embryos and immature inflorescences of *Hordium vulgare* L. *Plant Physiol.,* 121: 159-169.

Vasil, V., F. Redway and I.K. Vasil. 1990. Regeneration of plants embryogenic suspension culture protoplast of wheat (*Triticum aestivum* L.). *Bio/Tech.,* 8: 429-434.

Vasil, V., V. Srivastava, A.M. Castilllo, M.E. Fromm and I.K. Vasil. 1993. Rapid production of transgenic wheat by direct bombardment of cultured immature embryos. *Bio/Tech.,* 11: 1553-1558.

Viertel, K. and D. Hess. 1996. Shoot tips of wheat as an alternative source for regenerable embryogenic callus cultures. *Plant Cell, Tiss. and Org. Cult.,* 44: 183-188.

Yan, C.J. and Q.H. Zhao. 1982. Callus induction in plantlet regeneration from leaf blade of *Oryza sativa* L. subsp indica. *Plant Sci. Lett.,* 29: 175-182.

Zheng, M.Y. and C.F. Konzak. 1999. Effect of 2, 4-dichlorophenoxyacetic acid on callus induction and plant regeneration in anther culture of wheat (*Triticum aestivum* L.). *Plant Cell Rep.,* 19: 69-73.

Zhou, H., Y. Zheng and C.F. Konsak. 1991. Osmotic potential of medium affecting green plant percentage in wheat anther culture. *Plant Cell Rep.,* 10: 63-66.

(Received for publication 18 June 2009)