EFFECT OF GENOTYPES AND THEIR INTERACTION WITH 2,4 DICHLOROPHENOXY ACETIC ACID (2,4-D) LEVELS ON WHEAT'S IMMATURE EMBRYO CULTURE RESPONSE

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Wheat is one of the most important cereal crops in the world. Therefore, it has been extensively investigated with respect to plant regeneration from *in vitro* culture. Shoot regeneration is of crucial importance in the realization of the potential of cell and tissue culture techniques for plant improvement (Purnhauser *et al*., 1987). Tissue culture technique also offers creation of variation through somaclonal and gametoclonal variations. These variations could be exploited for crop improvement program. Therefore, plant regeneration from callus cultures could provide useful new germplasm for plant breeding program. *In vitro* regeneration of wheat is possible from different explants such as mature and immature embryos, seeds, endosperm, leaves, shoot bases and root tips (Sarker and Biswas, 2002). Among them the immature embryo was reported as the best organ for callus induction and shoot regeneration (Arzani and Mirodjagh, 1999; Hou *et al*., 1997; Wu *et al*., 2002; Pellegrineschi *et al*., 2004). Callus induction and regeneration potential are affected by cultivars, explants and carbohydrate 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 and Vasil, 1982 and 1983; He *et al*., 1986; Armstrong *et al*., 1987; Ben Amer *et al*., 1992; Machii *et al*., 1998).

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; Deambrogio and Dale, 1980; Lu *et al*., 1982; Thomas and Scott, 1985; Fladung and Hesselbach, 1986; Kamil, 2002; Sikandar *et al*. 2007; Rahman *et al*., 2008; Afzal *et al*., 2010). In most of these studies, an optimal concentration of 2,4-D for callus induction and growth was investigated which varied with the species.

Thus, this investigation aimed to study the effects of genotype, levels of 2,4-D and their interaction on immature embryo induction in wheat. In addition, to partition the phenotypic variances presented in this process to its components and subsequently, estimation of heritability percentages for *in vitro* traits.

MATERIALS AND METHODS

Plant material

In this investigation six wheat varieties were used. Three varieties were Sakha 8, Sakha 93 and Sakha 94 belong to the species (*Triticum aestivum*, L.) and another three varieties were Sohag 3, Benysweif 4 and Benysweif 5 belongs to the species (*Triticum durum* L.). All these varieties were supplied by Field Crops Research Institute (FCRI), Agriculture Research Center (ARC), Giza, Egypt.

Embryo culture procedure

During winter season (2010/2011), seeds of these varieties were cultivated at the Experimental Station, Faculty of Agriculture, Mansoura University, Egypt. At the flowering stage, all varieties were bagged to keep them self-pollinated in order to increase seeds. The flowing season 2011/2012, selfed seeds from these varieties were sown. Young spikes were collected about 15 days post anthesis from each genotype. Immature caryopses were surface sterilized under sterile conditions by immersing them for one minute in 75% ethanol followed by immersion in 0.1% mercuric chloride solution with 2 drops of Tween 20 as a wetting agent for 20 minutes. Then, it was rinsed three times in sterile double distilled water. Immature embryos were excised from each grain aseptically and cultured with the scutellm side up on induction medium under sterile conditions. The induction medium used in this study was MS medium, which recommended by Murashige and Skoog (1962) containing 3% sucrose and supplemented with three different concentrations of 2,4-dichlorophenoxy acetic acid (2,4-D) as the following:

A: MS medium with 2 mg/l 2,4-D

B: MS medium with 4 mg/l 2,4-D

C: MS medium with 6 mg/l 2,4-D

The cultures were incubated in darkness at 25°C ± 2°C for four weeks. Then, the total number of responding embryo, the total number of calli and the fresh weight of calli were recorded. The produced calli were transferred to regeneration medium with 1 mg/l 1-Naphthylacetic acid (NAA) and 0.5 mg/l kinetin. The cultures were kept under 16-hour's illumination (fluorescent light) at 22°C ± 2°C for four weeks. The regenerable calli, which have green shoot primordial were counted and transferred to the same regeneration medium under the same condition for shoot development for four weeks. When the regenerated plants were about 5-8 cm length and have normal roots and shoots were carefully pulled up from the containers and their roots were washed with tap water to remove the remained parts of medium. Then, the total number of plantlet, plant weight, plant length, root length and number of tiller were recorded. These plants were sown in 12 cm plastic pots and kept in a growth room at 20°C, R.H 80-90% and 16 h photoperiod for four weeks.

Experimental design

The experiments were designed as a complete randomized for the wheat gen-

otypes used under three different levels of 2,4-D. Each treatment was replicated three times. The specific MS medium for each treatment was distributed into culture Petri dishes (12 cm inner diameter); each one contained 20 ml of medium. The culture Petri dishes were autoclaved at 121°C, 15 pis for 20 min. One Petri dish containing 10 sterilized immature embryos is considered as one of experimental unit.

RESULTS AND DISCUSSION

As can be seen in Table (1), test of significance on the mean squares of genotypes at the three levels of 2,4-D, which indicated the presence of significant differences between these genotypes for all *in vitro* traits with respect to the three 2,4-D levels except for responding embryos and number of regenerated plants at B level as well as number of tillers per plant at A and B levels. However, replications of mean squares were not significant in most of occasions, indicating that there was no difference between spikes, which were collected from each genotype of immature embryos. In addition, the data which were obtained from the three 2,4-D levels for varieties were set up in a combined analysis of variance and the obtained results are presented in Table (2). Significance testes on the mean squares of genotype were highly significant for all studied *in vitro* traits. These traits were: responding embryos (R.E.) (Fig. 1), callus fresh weight (C.F.W.), number of calli (N.C.), number of green point (G.P.) (Fig. 2), number of plants (N.P.) (Fig. 3), plant weight (P.W.), plant length (P.L.), Root length (R.L.) (Figs 4 and 5) and number of tillers (N.T.).

Means of six varieties for all *in vitro* traits at the three levels of 2,4-D (A, B and C) are presented in Table (3). The results showed that no specific variety was superior or inferior for all studied traits at different levels of 2,4-D. However, the six varieties at level A, regarding durum wheat, the greatest means of responding embryo, plant weight and number of tiller were observed in Sohag 3 with means of 0.957, 0.858 and 2.020, respectively, but greatest mean of plant weight was observed in Benysweif 4, while the greatest means values for callus weight, number of calli, number of green point, number of plants, plant length and root length were observed in Benysweif 5. Regarding aestivum wheat, the greatest values for responding embryo, number of plants and number of tiller were observed in Sakha 93 with means of 0.833, 0.277 and 1.567, respectively and the greatest values for callus weight and number of calli were observed in Sakha 94 with means 0.083 and 6.633, respectively. The greatest values for number of green point, plant weight, plant length and root length were detected in Sakha 8 with means of 2.643, 0.784, 17.083 and 4.847, respectively. Although, the greatest values for responding embryo and number of tiller were recorded in Sohag 3 with means of 0.957 and 2.020, respectively, the greatest values for plant weight were observed in Sohag 3 and Benysweif 4 with the same mean value which equal to 0.858. In addition, the greatest values for callus weight, number

of plants, plant length and root length were observed in Benysweif 5 with means of 0.111, 0.553, 22.760 and 7.230, respectively. Regarding the number of calli and number of green point Sakha 8 seemed to be the best variety with means of 6.633 and 2.643, respectively. On the other hand, the lowest mean values for responding embryo and number of calli were observed in Benysweif 4 with means of 0.677 and 3.177, respectively. The lowest means value for callus weight was recorded in Sakha 8 with mean 0.056 and the lowest means value for number of green point was observed in Sohag 3 with mean 0.567, whereas lowest means values for number of plants, plant length and number of tiller were observed in Sakha 94 with means of 0.087, 11.417 and 1.337, respectively. Sakha 93 gave the lowest means values for plant weight and root length with means of 0.408 and 1.893, respectively.

At level B, regarding durum wheat (Sohag 3, Benysweif 4 and Benysweif 5), although the greatest mean values for responding embryos, callus weight, number of green point, number of plants, plant weight, plant length and root length were recorded in Benysweif 5, while the greatest mean value for number of tiller was recorded in Benysweif 4 but Sohag 3 was the greatest value for number of calli. For aestivum wheat (Sakha 8, Sakha 93 and Sakha 94) the greatest values for responding embryo, number of calli, plant weight, plant length and root length were recorded in Sakha 94, while the greatest values for number of green points, number of plants and number of tiller were recorded in Sakha 8. Whereas, Sakha 93 gives the greatest value for callus weight. The greatest overall values for responding embryo and plant weight were recorded in Sakha 94, while the greatest values for callus weight, number of green points, number of plants, plant length and root length were recorded in Benysweif 5 and the greatest value for number of tiller was recorded in Benysweif 4. Whereas, the greatest value for number of calli was recorded in Sohag 3. On the other hand, the lowest overall for responding embryo and number of green points were observed in Sohag 3. While, Sakha 8 was recorded the lowest value for callus weight but Sakha 93 were the lowest values for number of calli, plant weight, plant length, root length and number of tillers per plant. Whereas, Sakha 94 was the lowest value for number of plants. In general these values ranged from 0.943 to 1.00 for Sohag 3 and Sakha 94, respectively with respect to responding embryos, while it ranged from 0.480 to 3.243 in the case of regeneration ability (green point percentages) for Sohag 3 and Benysweif 5, respectively. These findings indicated that the variety Sohag 3 was the inferior one among the studied varieties for immature embryos culture purpose with respect to level B.

Regarding level C, although the greatest mean values for responding embryo, number of green points, and root length were recorded in Benysweif 5 with percentages values of 1.00, 4.04 and 7.36, respectively, Benysweif 4 was the best for number of plants, plant weight, plant

length and number of tillers with percentages values of 0.29, 0.94, 20.24 and 2.47, respectively. Whereas, Sohag 3 showed the same percentage value with Benyseweif 5 in the case of responding embryos and it was the best one for callus weight with percentage value of 0.16. On the other hand, Sakha 93 and Sakha 94 seemed to be the inferior varieties for regeneration ability which recorded the lowest percentages values for number of plants, plant weight, plant height, root length and number of tillers per plant with respect to MS basal medium supplemented with 6 mg/l 2,4-D (C level).

Since, these genotypes which included (*Triticum aestivum* L.) and (*Triticum durum* L.) varieties gave different performance with different 2,4-D levels as observed in Table (4) for most of studied *in vitro* traits. Therfore, the combined data over the three 2,4-D levels could be more precise to present information concerning the behavior of these genotypes. For that, the average means of six varieties were determined from the data combined over the three levels of 2,4-D and the obtained results are shown in Table (4). The results revealed that there are significant differences between paired of means in most of studied traits. The results pointed out that the greatest percentages values for responding embryo, green points, number of plantlets, plant length and root length were recorded for Benysweif 5 with means of 0.97, 2.90, 0.39, 20.69 and 8.00 for these traits, respectively. In addition, Sohag 3 was the best variety for responding embryos, callus weight and number of calli, which recorded percentages values of 0.967, 0.133 and 8.500, respectively. Whereas, the greatest values for plant weight and number of tiller were observed in Benysweif 4 with means of 0.823 and 1.862, respectively. On the other hand, Sakha 94 followed by Sakha 93 appeared to be the wariest varieties for immature embryos culture purpose, which gave poorest response to this process over all the three levels of 2,4-D concentrations used in this study.

In general, from the previous results it could be observed that durum varieties are more suitable for immature embryo culture compared to *aestivum* varieties with respect to the genotypes used in this study. These results could be in line with the results obtained by Ahmed and Allam (2003). They found that the hybrid pro-embryos of hexaploid wheat x rye were much more responsive than those of tetraploid x rye. Nasircilar *et al*. (2006) reported that among the *T. aestivum* cultivars, Yakar had the highest regeneration capacity in two induction media. In *T. durum* cultivars, Kiziltan gave the highest regeneration capacity on MS+2,4-D medium and Yilmaz gave the highest regeneration capacity on MS+NAA medium.

Owing to the 2,4-D levels effect as observed earlier, it could be more informative to average the performances of all studied genotypes over each level. Therefore, the average means of the three 2,4-D levels over all genotypes for all *in vitro* studied traits are presented in Table

(5). The results indicated that the greatest means values were observed in the level (C) for all studied traits except number of green points. These findings revealed that the addition of 6 mg/l 2,4-D to MS nutrient medium and 1 mg/l NAA and 0.5 mg/l Kinetin in regeneration medium could be the best concentrations for immature embryo culture purpose in wheat. In this respect, Hassanien *et al*. (2000) reported that the highest concentration of the auxin (6 mg/l) gave the best callus induction and plantlet regeneration in wheat. Sarkar and Biswas (2002) indicated that the MS medium supplemented with 6.0 mg/l of 2,4-D showed the best response for callus induction from mature wheat embryos. Rahman *et al*. (2008) found that maximum number of calli were produced on MS medium supplemented with 6.0 mg/l of 2,4-D. But comparatively larger calli were produced on MS medium supplemented with 4.0 mg/l of 2,4-D. Mahmood *et al.* (2012) indicated that cultivars AS-2002 and GA-2002 produced maximum number of calli on induction media comprising 4 mg/l 2,4-D; while, cv. Chakwal-50 performed best at 6 mg/l of 2,4-D while, Shah *et al*. (2003) found that good callus formation was obtained on MS medium containing 3.5 mg/l 2,4-D, Raziuddin *et al.* (2010) found that MS medium containing 2 mg l^{-1} 2, 4-D produced the greatest number of calli. Whereas, El-Wafa and Ismail (1999) reported that the application of 2 mg 2,4-D/litre gave the greatest response for all studied characters.

The genetic variation and heritability in broad (H_b%) sense were estimated within each 2,4-D levels for all *in vitro* studied traits and the obtained results are presented in Table (6). The results revealed that the magnitude of genetic variation was positive for all *in vitro* traits at the three levels of 2,4-D. The results showed that genetic variation was high for all traits with respect to the three levels of 2,4-D (2 mg, 4mg and 6 mg/l) except for responding embryos and number of plants at level B (4 mg/l) and number of tillers/plant at levels A and B. These results confirmed by the values of heritability, which ranged from 66.67 to 98.7% for callus weight at level A and plant weight at level C, respectively. Moreover, the results also showed that heritability in broad sense (H_b%) was high (more than 85%) for all traits in the high concentration of 2,4-D (6 mg/l). The results showed that increasing concentrations of 2,4-D led to increase heritability for most *in vitro* traits. Thus, it could be more precise to estimate these parameter from the data combined over three levels of 2,4-D concentrations. Therefore, the relative magnitudes of these parameters were estimated for all studied *in vitro* traits from the combined data over the three levels and the obtained results are shown in Table (7). The results revealed that the genetic variations were high and positive for most of studied traits. This finding is emphasized by the heritability values, which were more than 60% for all studied traits except for responding embryos, plant weight and number of tillers/plant. In addition, the values of genetic by levels interaction variations were high and positive in all studied *in vitro* traits, especially in the

cases of responding embryos, plant weight and number of tillers/plant. These findings explain the low values of heritability in these traits as well as the genes control these traits are highly affected by media composition. In this respect, El-Wafa (1999) found that the genetic variance was extremely large in magnitude relative to environmental variance for the studied traits. In addition, the traits showed high heritability, El-Wafa (1999) reported that the genetic variance relative to environmental variance was large in magnitude for studied traits. Heritability (broad-sense) and phenotypic and genotypic coefficients of variability were high for fresh weight of callus at one month and increasing weight of callus at one month. Lange *et al*. (1998) found that estimated genetic variance and heritability in the broad sense had very distinct values among the crosses of the traits.

Genotypic and phenotypic correlation

Genotypic (r_g) and phenotypic (r_{ph}) correlation among different pairs of the traits were studied from the data combined over the three levels of 2,4-D concentrations and the obtained results are shown in Table (8). The results revealed that both phenotypic (above diagonal) and genotypic (below diagonal) correlation coefficient values were close with respect to most of studied traits. Although, most of pairs of traits exhibited positive or negative but non-significant correlation coefficient values, number of calli appeared to be significantly correlated with responding embryos, callus weight, green points and number of plants. This trait (number of calli) is genetically correlated with responding embryos, callus fresh weight, green point and number of plants and the coefficient values were 0.88, 0.89, 0.83 and 0.84, respectively. Owing to this results, number of calli could be used as indicator trait for selection the genotypes which suitable for immature embryos culture purpose in wheat.

SUMMARY

The main objective of this investigation was to study the effects of genotype, levels of 2,4-D (2,4-dichlorophynoxyacetic acid) concentrations and their interaction on immature embryo induction in wheat. In addition, to partition the phenotypic variances presented in this process to its components and subsequently, estimating the heritability percentages for *in vitro* traits. The genetic materials used in this study were six varieties. Three varieties belong to the species (*Triticum aestivum* L.) and another three varieties belong to the species (*Triticum durum* L.). These genotypes were *in vitro* evaluated for immature embryos culture ability at three levels of 2,4-D. The results showed the presence of significant differences among genotypes at the three levels of 2,4-D for all *in vitro* traits except for responding embryos and number of regenerated plants at B level as well as number of tillers per plant at A and B levels. Furthermore, levels and genotype x levels interaction of mean squares were highly significant with respect to all the studied *in vitro* traits except in a few cases. This

indicates that these genotypes gave different response at different 2,4-D levels. The results revealed that greatest percentages values for responding embryo, green points, number of plantlets, plant height and root length were recorded for Benysweif 5. In addition, Sohag 3 was the best variety for responding embryos, callus weight and number of calli. Whereas, the greatest values for plant weight and number of tiller were observed in Benysweif 4. Therefore, durum varieties are more suitable for immature embryo culture compared to *aestivum* varieties. Furthermore, the results revealed that the genetic variation were high and positive for most of studied traits. This finding is emphasized by the heritability values, which were more than 60% for all studied traits except for responding embryos, plant weight and number of tillers/plant. In addition, the values of genetic levels of interaction variations were high and positive in all studied *in vitro* traits. This finding explains the low values of heritability in these traits as well as the genes control these traits are highly affected by media compositions.

REFERENCES

Ahmed, K. Z. and H. Z. Allam (2003). Response of intergeneric hybrids of Egyptian wheat with rye to *in vitro* techniques. African Crop Science Conference Proceedings, 6: 98-102.

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

Arzani, A. and S. S. Mirodjagh (1999). Response of durum wheat cultivars to immature embryo culture, callus induction and *in vitro* salt stress. Plant Cell, Tissue & Organ Culture, 58: 67-72.

Afzal, A., H. Rashid, M. H. Khan, Z. Chaudhry and S. A. Malik (2010). High frequency regeneration system optimization for wheat cultivar Inqilab-91. Pak. J. Bot., 42: 1857-1862.

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.

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.

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.

El-Wafa, A. A. A. (1999). Response of immature embryos *in vitro* regeneration of some wheat (*T. aestivum*) genotypes under different osmotic stress of mannitol. Assiut Journal of Agricultural Sciences, 30: 25-34.

El-Wafa, A. A. A. and A. E. A. Ismail (1999). Callus induction and plant regeneration from culture of immature embryos of spring wheat. Assiut Journal of Agricultural Sciences, 30: 13-23.

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

Hassanien, S. H., M. S. Abdel-Hady, F. M. El-Domyati and A. I. Tayeb (2000). Wheat pedicle as an alternative source for plant regeneration. Arab J. Biotech., 3: 209-216.

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.

Hou, B., H. Yu and S. Teng (1997). Effects of low temperature on induction and differentiation of wheat calluses. Plant Cell Tiss. Org. Cult., 49: 35-8.

Kamil, H. (2002). Wheat immature embryo culture for embryogenic callus induction. Journal of Biological Sciences, 2: 520-521.

Lange, C. E., L. C. Federizzi, F. I. F. Carvalho, A. L. C. Dornelles and C. L. Handel (1998). Genetic of *in vitro* organogenesis and precocious germination of wheat embryos. Genet. Mol. Biol., 21: 1-15.

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

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.

Mahmood, I., A. Razzaq, Z. U. Din Khan, I. A. Hafiz and S. Kaleem (2012). Evaluation of tissue culture responses of promising wheat (*Triticum aestivum* L.) cultivars and development of efficient regeneration system. Pak. J. Bot., 44: 277-284.

Murashige, T. and F. Skoog (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Physiol. Plant, 15: 473-497.

Nasircilar, A. G., K. Turgut and K. Fiskin (2006). Callus induction and plant regeneration from mature embryos of different wheat genotypes. Pak. J. Bot., 38: 637-645.

Ozias-Akins, P. and I. K. Vasil. (1982). Plant regeneration from cultured

immature embryos and inflorescences of *Triticum aestivum* L. (wheat) evidence for somatic embryogenesis. Protoplasma, 110: 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.

Pellegrineschi, A., R. M. Brito, S. McLean and D. Hoisington (2004). Effect of 2,4-Dichlorophenoxyacetic Acid and NaCl on the Establishment of Callus and Plant Regeneration in Durum and Bread Wheat. Plant Cell, Tissue and Organ Culture, 77: 245-250.

Purnhauser, L., P. Medgyesy, M. Czako, P. J. Dix and L. Marton (1987). Stimulation of shoot regeneration in *Triticum aestivum* and *Nicotiana plumbaginifolia via* tissue cultures using the ethylene inhibitor $AgNO_3$. Plant Cell Reports, 6: 1-4.

Rahman, M. M., A. K. M. Shamsuddin and U. Asad (2008). *In vitro* regeneration from mature embryos in spring wheat. Int. J. Sustain. Crop Prod., 3: 76-80.

Raziuddin, J. Bakht, Z. A. Swati, M. Shafi, F. Ullah and M. Akmal (2010). Effect of cultivars and culture medium on callus formation and plant regeneration from mature embryos of wheat (*Triticum aestivum* L.). Pak. J. Bot., 42: 639-652.

Sarker, R. H. and A. Biswas (2002). *In vitro* plantlet regeneration and *Agrobacterium* mediated genetic transformation of wheat. Plant Tissue Cult., 12: 155-165.

Shah, M. I., M. Jabeen and I. Ilahi (2003). *In vitro* callus induction, its proliferation and regeneration in seed explants of wheat (*Triticum aestivum* L.) var. lu-26s. Pak. J. Bot., 35: 209-217.

Sikandar, W. A., I. Khan and I. Munir (2007). Optimization of *In vitro* conditions for callus induction proliferation and regeneration in wheat (*Triticum aestivum*) cultivars. Bio/Technol., 6: 420-425.

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.

Wu, B. H., Y. L. Zheng, D. C. Liu and Y. H. Zhou (2002). Trait correlation of immature embryo culture in bread wheat. Plant Breeding, 121: 1-5.

Table (1): Analysis of variance and the mean squares of varieties at the three levels of 2,4-D.

S.O.V		d.f	R.E.	C.F.W. (g)	N.C.	G.P.	N.P.	P.W. (g)	P.L. (cm)	R.L. (cm)	N.T.
Replicates	A	2	0.003	0.001	0.203	0.371	0.013	0.011	0.799	0.444	0.112
	B		0.003	0.0004	1.948	0.190	0.031	0.202	33.691*	10.488**	0.222
	C		0.002	0.0004	2.679**	0.039	0.001	0.017	1.895	0.598	0.071
Genotypes	A	5	0.033*	0.001*	5.478**	2.174**	0.093**	0.091*	57.999*	11.102*	0.161
	B		0.001	0.002**	11.643**	3.678**	0.087	0.354**	53.471**	23.270**	0.023
	C		0.007**	0.004**	11.926**	4.469**	0.036**	0.462**	222.584**	31.974**	2.663**
Error	A	10	0.008	0.0003	0.403	0.194	0.011	0.020	11.162	3.196	0.093
	B		0.001	0.0002	0.605	0.450	0.049	0.056	8.050	1.182	0.136
	C		0.001	0.0002	0.351	0.164	0.001	0.006	4.983	1.183	0.051

Note: *, ** Significant at 0.05 and 0.01 levels of probability, respectively.
A: MS medium with 2 mg/l 2,4-D B: MS medium with 4 mg/l 2,4-D
C: MS medium with 6 mg/l 2,4-D R.E: responding embryos, C.F.W: callus fresh weight, N.C: number of calli, G.P: number of green point, N.P: number of plants, P.W.: plant weight, P.L.: plant length, R.L.: Root length and N.T: number of tillers

Table (2): Combined analysis of variance and the mean squares of genotypes, levels and their interactions for all *in vitro* traits.

S.O.V	d.f	R.E.	C.F.W. (g)	N.C.	G.P.	N.P.	P.W. (g)	P.L. (cm)	R.L. (cm)	N.T.
Levels (L)	2	0.106**	0.0048**	72.575**	0.482	0.209**	0.4249**	201.855**	10.574**	1.411**
Reps / L	6	0.003	0.0003	1.610	0.200	0.015	0.0768	12.128	3.843	0.135
Genotypes (G)	5	0.017**	0.0055**	17.368**	7.274**	0.152**	0.3410**	250.700**	55.129**	1.308**
G x L	10	0.012**	0.0004	5.840**	1.524**	0.032	0.2832**	41.678**	5.609**	0.769**
Error	30	0.003	0.0002	0.453	0.269	0.020	0.0274	8.065	1.854	0.093

Note: *,** Significant at 0.01 levels of probability.
R.E: responding embryos, C.F.W: callus fresh weight, N.C: number of calli, G.P: number of green point, N.P: number of plants, P.W: plant weight, P.L: plant length, R.L: Root length and N.T: number of tillers

Table (3): Mean performance of varieties for all *in vitro* traits at the three levels of 2,4-D (A, B and C).

Genotypes	Responding embryo			Callus fresh weight (g)			Number of calli			Number of green point			Number of plants		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Sohag 3	0.957^{a}	0.943^{a}	1.000^{a}	0.101^{a}	0.134^{ba}	0.164^{a}	5.233^{b}	10.990^{a}	9.277^{cb}	0.567^{d}	0.480^{c}	0.423^{d}	0.490^{a}	0.300^{a}	0.130^{cb}
Benysweif 4	0.677^{c}	0.957^{a}	0.967^{a}	0.090^{ba}	0.111^{bc}	0.144^{a}	3.177^{c}	6.887^{cb}	6.567^{d}	0.857^{dc}	1.103^{cb}	1.863^{cb}	0.290^{b}	0.410^{a}	0.287^{a}
Benysweif 5	0.933^{ba}	0.967^{a}	1.000^{a}	0.111^{a}	0.139^{a}	0.143^{a}	5.757^{ba}	8.233^{b}	10.013^{b}	1.443^{bc}	3.243^{a}	4.043^{a}	0.553^{a}	0.437^{a}	0.177^{b}
Sakha 8	0.800^{bac}	0.967^{a}	0.900^{b}	0.058^{b}	0.075^{d}	0.081^{b}	4.720^{b}	5.957^{c}	6.777^{d}	2.643^{a}	3.200^{a}	2.267^{b}	0.200^{b}	0.143^{a}	0.100^{c}
Sakha 93	0.833^{bac}	0.957^{a}	0.977^{a}	0.083^{ba}	0.100^{d}	0.097^{b}	3.377^{c}	5.887^{c}	8.253^{c}	2.500^{a}	1.937^{b}	1.247^{c}	0.277^{b}	0.077^{a}	0.0001^{d}
Sakha 94	0.767^{bc}	1.000^{a}	0.890^{b}	0.083^{ba}	0.096^{d}	0.090^{b}	6.633^{a}	6.310^{c}	11.767^{a}	1.913^{ba}	1.813^{b}	1.567^{cb}	0.087^{b}	0.070^{a}	0.0001^{d}

Genotypes	Plant weight (g)			Plant length (cm)			Root length (cm)			Number of tiller		
	A	B	C	A	B	C	A	B	C	A	B	C
Sohag 3	0.858^{a}	0.636^{cb}	0.744^{b}	17.743^{ba}	14.343^{bc}	13.627^{b}	3.810^{bac}	3.160^{cd}	1.880^{cb}	2.020^{a}	1.333^{a}	1.500^{b}
Benysweif 4	0.858^{a}	0.675^{cb}	0.935^{a}	20.253^{a}	17.660^{ba}	20.240^{a}	5.510^{ba}	5.553^{b}	6.887^{a}	1.653^{ba}	1.467^{a}	2.467^{a}
Benysweif 5	0.752^{a}	0.916^{b}	0.626^{cb}	22.760^{a}	22.553^{a}	16.770^{ba}	7.230^{a}	9.413^{a}	7.360^{a}	1.587^{ba}	1.350^{a}	1.113^{b}
Sakha 8	0.784^{a}	0.588^{cb}	0.588^{c}	17.083^{ba}	13.177^{bc}	13.070^{b}	4.847^{bac}	2.153^{d}	2.767^{b}	1.467^{ba}	1.400^{a}	1.193^{b}
Sakha 93	0.408^{b}	0.431^{c}	0.0001^{d}	12.397^{b}	10.233^{c}	0.0001^{c}	1.893^{c}	2.067^{d}	0.0001^{c}	1.567^{ba}	1.200^{a}	0.0001^{c}
Sakha 94	0.611^{ba}	1.397^{a}	0.0001^{d}	11.417^{b}	16.167^{b}	0.0001^{c}	2.837^{bc}	5.083^{cb}	0.0001^{c}	1.337^{b}	1.333^{a}	0.0001^{c}

Note: Means followed by the same letter in the same column are not significantly different at the 0.05 level of probability.
A: MS medium with 2 mg/l 2,4-D B: MS medium with 4 mg/l 2,4-D C: MS medium with 6 mg/l 2,4-D

Table (4): Mean performance of varieties for all *in vitro* traits from the combined data over the three levels of 2,4-D.

Genotypes	R.E.	C.F.W. (g)	N.C.	G.P.	N.P.	P.W. (g)	P.L. (cm)	R.L. (cm)	N.T.
Sohag 3	0.967^{a}	0.133^{a}	8.500^{a}	0.490^{d}	0.307^{ba}	0.746^{a}	15.238^{b}	2.950^{c}	1.618^{ba}
Benysweif 4	0.867^{b}	0.115^{b}	5.543^{b}	1.274^{c}	0.329^{ba}	0.823^{a}	19.384^{a}	5.983^{b}	1.862^{a}
Benysweif 5	0.967^{a}	0.131^{a}	8.001^{a}	2.910^{a}	0.389^{a}	0.765^{a}	20.694^{a}	8.001^{a}	1.350^{b}
Sakha 8	0.889^{b}	0.072^{d}	5.818^{b}	2.703^{a}	0.238^{bc}	0.653^{a}	14.443^{b}	3.256^{c}	1.353^{b}
Sakha 93	0.922^{ab}	0.093^{c}	5.039^{b}	1.894^{b}	0.118^{dc}	0.280^{b}	7.543^{c}	1.320^{d}	0.922^{c}
Sakha 94	0.886^{b}	0.090^{c}	0.237^{b}	1.764^{cb}	0.052^{d}	0.669^{a}	9.194^{c}	2.640^{c}	0.890^{c}

Note: Means followed by the same letter in the same column are not significantly different at the 0.05 level of probability.
R.E: responding embryos, C.F.W: callus fresh weight, N.C: number of calli, G.P: number of green point, N.P: number of plants, P.W: plant weight, P.L: plant length, R.L: Root length and N.T: number of tillers

Table (5): The 2,4-D levels averaged overall genotypes for *in vitro* traits.

Media	R.E.	C.F.W. (g)	N.C.	G.P.
MS+2.0 mg/l 2,4-D (A)	0.828^{b}	0.082^{b}	4.812^{c}	2.299^{c}
MS+4.0 mg/l 2,4-D (B)	0.966^{a}	0.111^{a}	7.377^{b}	1.127^{b}
MS+6.0 mg/l 2,4-D (C)	0.956^{a}	0.123^{a}	8.776^{a}	2.092^{a}
LSD $_{5\%}$	0.131	0.043	2.958	1.604

Note: Means followed by the same letter in the same column are not significantly different at the 0.05 level of probability.
R.E: responding embryos, C.F.W: callus fresh weight, N.C: number of calli,
G.P: number of green point, N.P: number of plants, P.W: plant weight,
P.L: plant length, R.L: Root length and N.T: number of tillers

Table (6): Estimation of relative magnitudes of different genetic parameters for *in vitro* traits at the three levels of 2,4-D (A, B and C).

Genetic Parameters	Responding embryo			Callus fresh weight (g)			Number of calli			Number of green point			Number of plants		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
σ^2g	0.0080	0.0000	0.0020	0.0002	0.0006	0.0013	1.6920	3.6790	3.858	0.6600	1.076	1.4350	0.0270	0.0130	0.0120
σ^2e	0.0027	0.0003	0.0003	0.0001	0.0001	0.0001	0.1343	0.2017	0.117	0.0647	0.150	0.0547	0.0037	0.0163	0.0003
σ^2ph	0.0107	0.0003	0.0023	0.0003	0.0007	0.0014	1.8263	3.8807	3.975	0.7247	1.226	1.4897	0.0307	0.0293	0.0123
Hb%	74.77	0.00	86.96	66.67	85.71	92.86	92.65	94.80	97.06	91.07	87.77	96.33	87.95	44.37	97.56

Genetic Parameters	Plant weight (g)			Plant length (cm)			Root length (cm)			Number of tiller		
	A	B	C	A	B	C	A	B	C	A	B	C
σ^2g	0.0240	0.0990	0.152	15.6120	15.1400	72.534	2.6350	2.363	10.2640	0.023	0.0380	0.871
σ^2e	0.0067	0.0187	0.002	3.7207	2.6833	1.661	1.0653	0.394	0.3943	0.031	0.0453	0.017
σ^2ph	0.0307	0.1177	0.154	19.3330	17.8230	74.195	3.7003	2.757	10.6580	0.054	0.0833	0.888
Hb%	78.18	84.11	98.70	80.75	84.94	97.76	71.21	85.71	96.30	42.59	45.62	98.09

A: MS medium with 2 mg/l 2,4-D B: MS medium with 4 mg/l 2,4-D C: MS medium with 6 mg/l 2,4-D

Table (7): Estimation of relative magnitudes of different genetic parameters for *in vitro* traits obtained from the combined data over the three levels.

Genetic parameters	R.E.	C.F.W. (g)	N.C.	G.P.	N.P.	P.W. (g)	P.L. (cm)	R.L. (cm)	N.T.
σ^2g	0.001	0.001	1.281	0.639	0.013	0.006	23.225	5.502	0.060
σ^2gL	0.003	0.000	1.796	0.418	0.004	0.085	11.204	1.252	0.225
σ^2e	0.001	0.0001	0.151	0.0897	0.0067	0.0091	2.6883	0.681	0.031
σ^2ph	0.003	0.0011	2.0307	0.868	0.021	0.0434	29.648	6.6003	0.166
Hb %	33.33	90.91	63.08	73.62	61.90	13.82	78.34	83.36	36.14

R.E: responding embryos, C.F.W: callus fresh weight, N.C: number of calli,
G.P: number of green point, N.P: number of plants, P.W: plant weight,
P.L: plant length, R.L: Root length and N.T: number of tillers

Table (8): Phenotypic (above diagonal) and Genotypic (below diagonal) correlations among pairs of *in vitro* traits.

	R.E.	C.F.W. (g)	N.C.	G.P.	N.P.	P.W. (g)	P.L. (cm)	R.L. (cm)	N.T.
R.E.		0.52	0.75	0.65	0.39	-0.06	-0.49	0.11	-0.08
C.F.W. (g)	0.57		0.86*	0.64	0.46	0.30	-0.16	0.30	0.03
N.C.	0.88*	0.89*		0.79	0.60	0.10	-0.39	0.23	-0.06
G.P.	0.77	0.71	0.83*		0.32	-0.01	-0.32	0.17	0.04
N.P.	0.80	0.68	0.84*	0.44		0.04	-0.29	0.15	0.07
P.W. (g)	-0.07	0.35	0.12	0.02	-0.07		-0.05	-0.16	0.12
P.H. (cm)	-0.57	-0.15	-0.42	-0.36	-0.37	-0.03		0.49	0.48
R.L. (cm)	0.31	0.44	0.34	0.26	0.17	-0.15	0.54		0.51
N.T.	-0.07	0.11	-0.03	0.09	0.06	0.06	0.51	0.60	

*,** Significant at 0.05 and 0.01 of probability, respectively.
R.E: responding embryos, C.F.W: callus fresh weight, N.C: number of calli,
G.P: number of green point, N.P: number of plants, P.W: plant weight,
P.L: plant length, R.L: Root length and N.T: number of tillers

Fig. (1): Responded embryo after four weeks.

Fig. (2): Green point after four weeks from calli.

Fig. (3): Green plantlets with a good root system on regeneration medium.

Fig. (4): Mature plant with root and shoot.

Fig. (5): Mature plant after transplanting to soil.