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Rapid Induction of Somatic Embryogenesis by 2,4-D in Leaf Base Cultures of Wheat (*Triticum aestivum* L.)

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Abstract

Wheat leaf base segments treated with 2,4-D (10 μM) for 24 h developed somatic embryos without an intervening callus when cultured on basal Murashige and Skoog's medium for less than 2 weeks. This response is auxin specific and genotype independent. In fact, vacuum infiltration of 2,4-D, although at a higher concentration (50 μM), in the basal leaf segments for 1 min, followed by culture on the basal medium, was sufficient to evoke the embryogenic response. In comparison to indirect somatic embryogenesis, this highly efficient system for direct embryogenesis not only curtails the time span for somatic embryogenesis by 15-20 days, it also obviates any secondary changes that may occur during dedifferentiation. The rapid induction of somatic embryogenesis in wheat leaf bases, under easily manipulable culture conditions, thus provides a unique opportunity to monitor the molecular events associated with auxin-mediated induction of somatic embryogenesis and also to study the associated developmental events.

Key words: 2,4-D (Auxin), Genotypes, Leaf base, Somatic embryogenesis (rapid induction), *Triticum aestivum*.

Abbreviations

BAP, 6-Benzylaminopurine; CPA, p-Chlorophenoxy-acetic acid; 2,4-D, 2,4-Dichlorophenoxyacetic acid; IAA, Indole-3-acetic acid; IBA, Indole-3-butyric acid; MS, Murashige and Skoog (1962); NAA, α-Naphthalene-acetic acid; 2,4,5-T, 2,4,5-Trichlorophenoxyacetic acid.

Introduction

Higher plants have the ability to differentiate whole plants from undifferentiated somatic cells in culture through the process of somatic embryogenesis. The potential of this unique developmental pathway has been exploited and several agronomic and horticulturally important species have been successfully regenerated (Merkle *et al.*, 1990; George, 1993; Thorpe, 1994; Vasil and Vasil, 1994; Young and Bhojwani, 1999). Although the process of somatic embryo induction from cells is not clearly understood, auxins, such as 2,4-dichlorophenoxyacetic acid (2,4-D), have been shown to play a vital role in initiation and maintenance of cell division (Dudits *et al.*, 1993, 1995) that eventually leads to the development of somatic embryos. It is generally believed that the continued presence of auxin is necessary for sustained cell divisions but for subsequent steps of maintenance or maturation of the somatic embryo, either lowering or complete removal of auxin is necessary (Nomura and Komamine, 1995; Raghavan, 2000; Chugh and Khurana, 2002).

Members of the Poaceae, including the cereals, that were once considered recalcitrant, can be regenerated via somatic embryogenesis. In wheat, somatic embryogenesis has been achieved from callus, cultured immature embryos, immature inflorescence, seed, and cell suspensions (Maheshwari *et al.*, 1995 and references cited therein), albeit with a low frequency. Limited information is also available on the regenerative capacity of somatic embryos derived from leaf explants of various wheat cultivars (Ahuja *et al.*, 1982; Zamora and Scott, 1983; Wernicke and Milkovits, 1984; Grossmann *et al.*, 1990; Rajyalakshmi *et al.*, 1991). However, in all these studies with various explants, somatic embryogenesis was invariably achieved through an intervening callus phase (indirect somatic embryogenesis). Despite the high morphogenic nature of the immature, undifferentiated basal segments, 2,4-

D plays an ambiguous role in stimulating growth in differentiated cells but suppressing growth at or in the vicinity of apical meristems (Wernicke *et al.*, 1986). The present study was thus undertaken to exploit the morphogenetic potential of the wheat leaf base explant and optimize the conditions for direct somatic embryogenesis (without callus formation).

Materials and Methods

Growth conditions

Seeds of hexaploid wheat (*Triticum aestivum*) cv. HD2329 were obtained from the Indian Agricultural Research Institute, New Delhi. Seeds were surface-sterilized in 3% sodium hypochlorite for 45 min, rinsed three times with sterile-distilled water, and inoculated on MS (Murashige and Skoog, 1962) medium supplemented with 0.8% Difco Bacto agar. Plants were allowed to grow under a daily schedule of 16 h light and 8 h dark. Light was provided by fluorescent lamps (Philips, TL 40W/54) at an irradiance of 5.27 Wm^{-2} and temperature maintained at 26±2 ℃ and 24±1 ℃ during light and dark phase, respectively. Data represent the mean of 2 to 4 independent experiments, each consisting of 30-40 explants.

Leaf base cultures

Fifteen-day-old *in vitro* raised seedlings were used. The leaf sheath and the primary leaf were discarded. The basal portions (about 5 mm in length) of the second and third leaves were cultured on MS medium with various growth regulators in 9 cm Petri dishes for one day in the dark and subsequently transferred to MS basal medium and incubated for 9 more days in the dark before the observations were recorded. For time-course experiments, the leaf base explants were treated with 2,4-D for the stipulated period either on agar-gelled medium or vacuum-infiltrated in liquid medium (for short-term treatments) and subsequently transferred to agar-gelled MS basal medium.

Scanning electron microscopy

Leaf base explants showing different stages of somatic embryogenesis were fixed in 2% glutaraldehyde at room temperature for 4 h and then washed three times with 0.1 M sodium phosphate buffer (pH 7). Explants were then dehydrated through an ascending series of acetone. The samples were critical point-dried, coated with silver, and subjected to scanning electron microscopy (501B Philips, The Netherlands).

Results and Discussion

Grasses have a basal leaf meristem and leaf segments cultured with this meristematic region exhibit high morphogenic response (Wernicke and Milkovits, 1984). In wheat too the basal region has been observed to be the most responsive region of the entire leaf blade (Ahuja *et al.*, 1982; Zamora and Scott, 1983, Wernicke and Milkovits, 1984; Rajyalakshmi *et al.*, 1991). In the present investigation, the basal leaf segments of wheat displayed high morphogenic competence and have been utilized for further experimentation.

Induction of somatic embryogenesis

Synthetic auxins are key factors in triggering embryogenic response in dicotyledonous (Sung *et al.*, 1984) as well as in monocotyledonous plants (Vasil, 1987); however, auxin is detrimental for the progression of embryogenesis. In the present investigation, embryogenesis was initiated from the leaf base by 2,4-D application. Leaf base explants cultured on a medium supplemented with 2,4-D (10 μM) dedifferentiated and developed callus. The callusing response was ca. 80% and when one-month-old callus was transferred to fresh medium containing a lower level of 2,4-D (0.1 or 0.5 μM), up to 20% regeneration could be achieved (data not presented). On microscopic examination, reversion of embryogenic masses to non-embryogenic tissue was observed, which may be responsible for this low regeneration potential.

Influence of a short-term 2,4-D treatment

To avoid any harmful effect of auxin on progression of somatic embryogenesis and improve the regeneration potential, basal leaf segments of wheat were subjected to a short-term 2,4-D treatment. When 2,4-D was provided for varying time periods and leaf segments transferred to the basal medium, even one-day treatment with 10 μM 2,4-D was effective in eliciting two types of morphogenic responses; cultures were analysed 10 days after 2,4-D treatment. The leaf base explants (**Fig. 1A**) either differentiated into compact, organized, globular masses (**Fig. 1B**), or into a soft, friable, white callus (**Fig. 1C**). These globular structures differentiated further and displayed various developmental stages of somatic embryogenesis, while the callus masses invariably differentiated into roots. Various stages of somatic embryogenesis, i.e. formation of globular embryoids (**Fig. 1B, F**), apical notch (**Fig. 1G**), emergence of the coleoptile (**Fig. 1D, H**) and, finally, germination of the bipolar somatic embryos

(**Fig. 1E, I**) were discernible. These somatic embryos could germinate, giving rise to complete plantlets, without any evident somaclonal variation, albinism, loss or reduction of fertility, or any other abnormal phenotype amongst the regenerants.

The growth regulator 2,4-D is generally provided for 3-4 weeks of culture and is known to cause a rapid induction of embryogenic proteins (Sung and Okimoto, 1981; Raghavan, 1997), initiate polyamine biosynthesis (Montague *et al.*, 1978; Fienberg *et al.*, 1984; Kakar *et al.*, 2000), and induce rapid cell divisions (Dudits, 1993). Induction of cell division has been observed as early as day 2 in carrot single cells (Nomura and Komamine, 1985) and day 3-4 in carrot (Fujimura and Komamine, 1980), wheat (Ozias-Akins and Vasil, 1987; Ryschka *et al.*, 1991) and barley (Ryschka *et al.*, 1991). Formation of proembryonal structures and somatic embryos could be observed as early as day 3 in carrot (Sung and Okimoto, 1981) and day 5 in wheat and barley (Ryschka *et al.*, 1991). In the present investigation, globular structures were seen

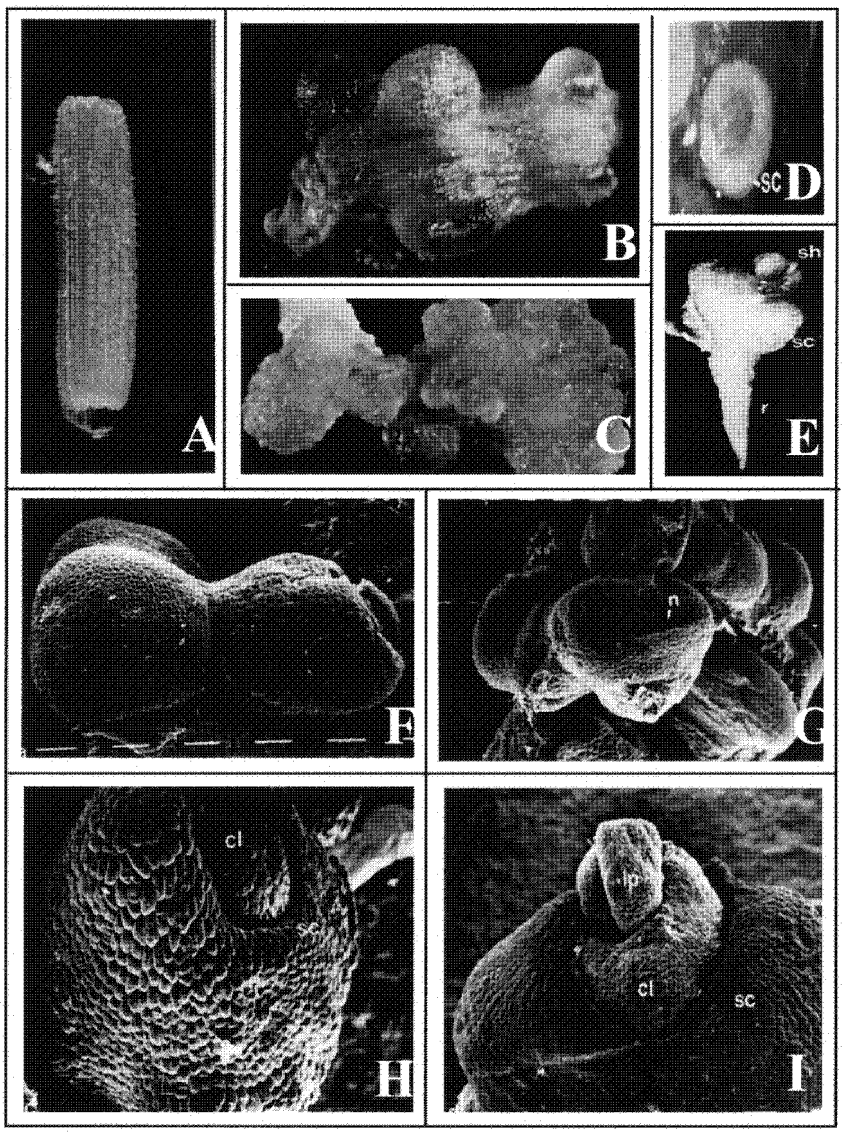

Fig. 1 Induction of somatic embryogenesis by 2,4-D in wheat leaf base explants. The explants were treated with (10 μM) 2,4-D for 24 h and transferred to the basal medium. For more details, see text. (A). Leaf base explant at the time of culture. (B). An embryogenic explant depicting globular embryos after 10 days in culture. (C). Another explant showing callusing after 10 days of culture. (D). Emergence of coleoptile from the scutellum in an embryo after 10-12 days of culture. (E). A bipolar germinating somatic embryo in a 15-day-old culture. (F to I) Scanning electron micrographs of the different stages of somatic embryogenesis in wheat leaf base explants cultured for 10 to 15 days. (F). Globular somatic embryos. (G). Embryos showing apical notch formation. (H). Emergence of coleoptile. (I). A germinating embryo. cl, coleoptile; lp, leaf primordia; n, notch; sc, scutellum. cl, coleoptile; r, root; sc, scutellum; sh, shoot. Bar represents 1 um.

Table 1 Effect of 2,4-D on induction of somatic embryos in leaf base cultures of wheat (*Triticum aestivum*) and % regeneration from the somatic embryos. For experimental details see text.

2,4-D Conc	No. of Explants Displaying SE	No. of Explants Regenerating Shoots[1)]	No. of Explants	% SE	% Regeneration
10 μM	106	33	26	31.13	78.78
50 μM	64	41	14	64.06	34.14
100 μM	61	47	11	77.04	23.40

[1)]On day 30 of Culture. SE: Somatic Embryogenesis

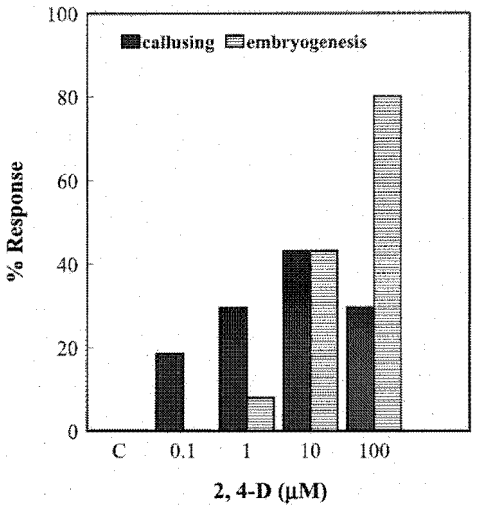

Fig. 2 Induction of somatic embryogenesis as influenced by the concentration of 2,4-D provided for the first 24 h of culture. The response was quantitated after 9 days of culture on the basal medium.

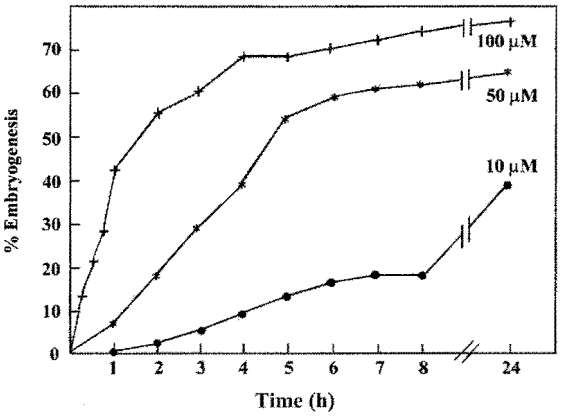

Fig. 3 Influence of 2,4-D provided for various durations on induction of somatic embryogenesis. After 2,4-D treatment, the explants were cultured on the basal medium for a total duration of 10 days.

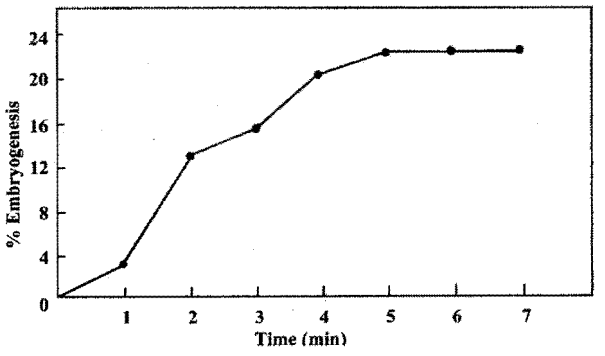

Fig. 4 Induction of somatic embryogenesis in the explants infilterated with 2,4-D (10, 50 or 100 μM) in the bathing medium for specified duration. The response was analysed in these explants after 10 days of culture on the basal medium.

2-3 days after 2,4-D treatment, which eventually lead to embryo differentiation. Histological examination of these globular structures ascertained the presence of cell divisions and embryos differentiation under the given experimental conditions.

When higher concentrations of 2,4-D (e.g. 100 μM) were provided for one day, nearly 75% of the cultures displayed embryogenesis (**Fig. 2**), but the subsequent regeneration of plantlets was lower than when 10 μM was provided (**Table 1**). In addition, at 100 μM 2,4-D, incubation of explants for even less than 1 h was found to be sufficient for the induction of somatic embryogenesis (**Fig. 3**); treatment of explants with 10 or 50 μM 2,4-D required a longer incubation period. This prompted us to provide 2,4-D for still shorter durations, and when 2,4-D was vacuum-infiltrated, induction of embryogenesis was observed even with an incubation period of few minutes (**Fig. 4**). This, to the best of our knowledge, is the shortest exposure to 2,4-D reported in literature for the induction of somatic embryogenesis. Usually, 2,4-D is provided for 2-3 weeks but in an earlier study on *Citrus*, 3% explants displayed embryogenesis with one day of 2,4-D treatment (Kamada and Harada, 1979). However, in the study by Kamada and Harada (1979), the manifestation of the response required 3 weeks of additional subculture on basal medium as against a 9-12 day incubation required in the present investigation. Similarly, in alfalfa, although 2,4-D (100 μM) can be provided for one hour, it requires 28 days of culture for the manifestation of response (Dudits *et*

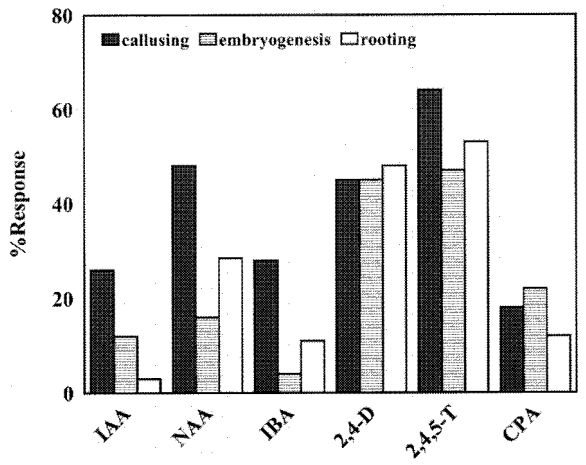

Fig. 5 Induction of somatic embryogenesis in wheat leaf bases exposed to various auxins for 24 h, followed by culture on the basal medium for 10 days.

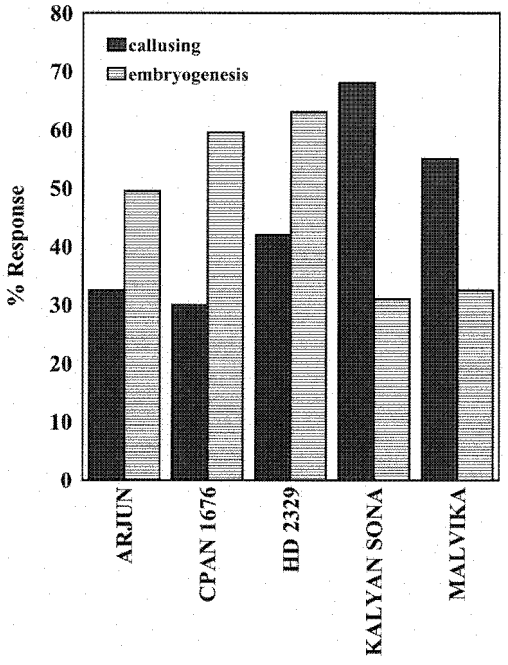

Fig. 6 Influence of genotype on short-term 2,4-D mediated induction of somatic embryogenesis in wheat leaf bases. The experimental protocol was similar to that described in legend to **Fig. 1**.

al., 1991). The rapid induction of somatic embryogenesis by 2,4-D in wheat leaf bases, in fact, finds a parallel with rapid auxin effects, such as increase in proton extrusion, respiration rate, decrease in the cytosolic pH and ATP/ADP ratios, and altered gene expression patterns, which occur or are initiated in the time span of 5-10 min (Brummell and Hall, 1987; Guilfoyle *et al.*, 1998, 1999; Thakur *et al.*, 2001). This report of rapid auxin induction of somatic embryogenesis may qualify as one of the rapid auxin effects in plants, although it may take a few more days for the eventual realization of the response in morphological terms.

Table 2 Effect of BAP (10 uM) on 2,4-D (10 μM) induced somatic embryogenesis in leaf bases of *Triticum aestivum* var. HD 2329. For experimental details, see text.

Treatment[1)]	% SE	% Rooting	% Callusing
BAP → BM	-	-	-
2,4-D → BM	37.8	15.6	30.4
BAP + 2,4-D → BM	5.0	4.7	27.8
2,4-D → BAP → BM	21.2	4.5	24.2
BAP → 2,4-D → BM	4.8	1.6	21.3

[1)]All treatments on hormone-supplemented media were for 24 h each, followed by transfer to basal medium for a total duration of 10 days. SE, Somatic embryogenesis.

Influence of other plant growth regulators

Among the various plant growth regulators tried, auxins alone were found to be effective for inducing somatic embryogenesis (**Fig. 5**), as use of either BAP, kinetin, 2-isopentenyl adenine, gibberellic acid, abscissic acid and 2-chloroethylphosphonic acid (each at 10 μM level), and spermine and putrescine (at 1 mM) did not elicit any morphogenic response (data not presented). Of the various auxins, the CPA derivatives, 2,4-D and 2,4,5-T, were most potent in inducing somatic embryogenesis (**Fig. 6**); our observation corroborates the earlier report of Kamada and Harada (1979) in carrot tissue cultures regarding the potency of the CPA derivatives.

Although in a majority of investigations auxins have been employed for induction of somatic embryogenesis, cytokinins too have proved to be effective in some instances (Thorpe, 1994). In cereals, however, the role of cytokinins in conferring morhogenic competence is quite equivocal (Bhaskaran and Smith, 1990). In the present investigation, however, BAP inhibited somatic embryogenesis when provided simultaneously, before or after 2,4-D treatment (**Table 2**). This is in conformity with earlier observations on carrot (Halperin and Wetherell, 1964; Kamada and Harada, 1979; Nomura and Komamine, 1985), citrus (Kochba and Spiegel-Roy, 1977) and orchard grass (Wenck *et al.*, 1988), where also BAP was found to be inhibitory for induction of somatic embryogenesis.

Influence of genotype

A relationship between plant genotype and *in vitro* response, particularly regeneration, has been well documented in crop plants including wheat (Lazar *et al.*, 1983; Galiba *et al.*, 1986; Mathias *et al.*, 1986; Wernicke and Milkovits, 1986; Carman

et al., 1987; Rajyalakshmi *et al.*, 1988). In the present investigation, both hexaploids (Arjun, CPAN 1676, HD 2329, Kalyan Sona) and a tetraploid (Malvika) were employed. In general, all genotypes displayed induction of somatic embryogenesis by 2,4-D (**Fig. 6**). However, the intensity of the response varied depending upon the genotype, which may well be attributed to the physiological status (including the endogenous hormone titre) of various explants at the time of culture. This view has also been advocated earlier for various other species including both monocots and dicots by Norstog (1970), Tran Thanh Van (1981), Krikorian *et al.* (1987) and Tran Thanh Van and Trinh (1990).

In conclusion, the present investigation has revealed the unusually high morphogenic potential of the basal leaf segment of various wheat cultivars. Of particular interest is its extreme sensitivity to short-term treatments of 2,4-D for rapid induction of somatic embryogenesis. During our experience of the last 6-7 years with this system, we have not noticed any somaclonal variation, albinism, etc amongst the regenerants. In addition, the results of this study should not only facilitate further detailed characterization of the induction and expression of somatic embryogenesis in plant cells but also pave the way for understanding the molecular mechanism of auxin action in general. Towards this end, we have made some attempts to identify the components that constitute auxin signal transduction pathway leading to changes in gene expression and eventually to more overt morphogenic responses. Employing various activators and inhibitors, our preliminary investigation has revealed that the Ca^{2+}-calmodulin signalling pathway may be operative in auxin-induced somatic embryogenesis (details to be published separately).

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References

Ahuja, P. S., Pental, D., Cocking, E. C., 1982. Plant regeneration from leaf base callus and cell suspension of *Triticum aestivum*. Z. Pflanzenzucht., **89**: 139-144.

Bhaskaran, S., Smith, R. H., 1990. Regeneration in cereal tissue. Crop Sci., **30**: 1328-1336.

Brummell, D. A., Hall, J. L., 1987. Rapid cellular response to auxin and the regulation of growth. Plant, Cell Environ., **10**: 523-543.

Carman, J. G., Jefferson, N. E., Campbell, F. W., 1987. Induction of embryogenic *Triticum aestivum* L. calli. I. Quantification of genotype and culture medium effects. Plant Cell, Tissue Organ Cult., **10**: 101-113.

Chugh, A., Khurana, P., 2002. Gene expression during somatic embryogenesis - recent advances. Curr. Sci., **83**: 715-730.

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

Dudits, D., Bogre, L., Bako, L., Dedeoglu, D., Magyar, Z., Kapros, T., Felfoldi, F., Gyorgyey, J., 1993. Key components of cell cycle control during auxin-induced cell division. In: Ormrod, J. C. and Francis, D. (Eds.): Molecular and Cell Biology of the Plant Cell Cycle, pp. 111-131. Kluwer Academic Publishers, Netherlands.

Dudits, D., Gyorgyey, J., Bogre, L., Bako, L., 1995. Molecular biology of somatic embryogenesis. In: Thorpe, T. A. (Ed.): In vitro Embryogenesis in Plants, pp. 267-308. Kluwer Academic Publishers, Netherlands.

Fienberg, A. A., Choi, J. H., Lubich, W. P., Sung, Z. R., 1984. Developmental regulation of polyamine metabolism in growth and differentiation of carrot culture. Planta, **162**: 532-539.

Fujimura, T., Komamine, A., 1980. The serial observation of embryogenesis in a carrot cell suspension culture. New Phytol., **86**: 213-218.

Galiba, G., Kovacs, G., Sukta, J., 1986. Substitution analysis of plant regeneration from callus culture in wheat. Plant Breed., **97**: 261-263.

George, E. F., 1993. Plant Propagation by Tissue Culture. Part 1. The Technology. Exegetics Limited, England.

Grossmann, K., Sawnbrey, E., Jung, J., 1990. Influence of growth retardants and ethylene-generating compounds on culture response of leaf explants from wheat (*Triticum aestivum* L.). J. Plant Physiol., **135**: 725-729.

Guilfoyle, T. J., 1999. Auxin-regulated genes and promoters. In: Hooykaas, P. J. J. *et al.* (Eds.): Biochemistry and Molecular Biology of Plant Hormones, pp. 423-459. Elsevier, Amsterdam, The Netherlands.

Guilfoyle, T. J., Hagen, G., Ulmasov, T., Murfett, J., 1998. How does auxin turn on genes? Plant Physiol., **118**: 341-347.

Halperin, W., Wetherell, D. F., 1964. Adventive embryogeny in tissue culture of the wild carrot. Am. J. Bot., **51**: 274-283.

Kakkar, R. K., Nagar, P. K., Ahuja, P. S., Rai, V. K., 2000. Polyamines and plant morphogenesis. Biol. Plant., **43**: 1-11.

Kamada, H., Harada, H., 1979. Studies on the organogenesis in carrot tissue culture. Z. Pflanzenphysiol., **91**: 255-266.

Kochba, J., Spiegel-Roy, P., 1977. The effect of auxins,

cytokinins and inhibitors on embryogenesis in habituated ovular callus of the "Shamouti" orange (*Citrus sinensis*). Z. Pflanzenphysiol., **81**: 283-288.

Krikorian, A. D., Kelly, K., Smith, D. L., 1987. Hormones in tissue culture and micropropagation, In: Davies, P. J. (Ed.): Plant Hormones and their Role in Plant Growth and Development, pp. 593-613. Martinus Nijhoff, Dordrecht.

Lazar, M. D., Collins, G. B., Vian, W. E., 1983. Genetic and environment effect on growth and differentiation of wheat somatic cell culture. J. Heredity, **74**: 353-357.

Maheshwari, N., Rajyalakshmi, K., Baweja, K., Dhir, S. K., Chowdhry, C. N., Maheshwari, S. C., 1995. *in vitro* culture of wheat and genetic transformation - retrospect and prospect. Crit. Rev. Plant Sci., **14**: 149-178.

Mathias, R. J., Boyd, L. A., Simpson, E. S., 1986. The interaction of genotype and culture medium on the tissue culture responses of wheat (*Triticum aestivum* L. em. Thell). Plant Cell, Tissue Organ Cult., **7**: 31-37.

Merkle, S. A., Parott, W. A., Williams, E. G., 1990. Applications of Somatic Embryogenesis and Embryo Cloning. In: Bhojwani, S. S. (Ed.): Plant Tissue Culture: Applications and Limitations, pp. 67-101. Elsevier, Amsterdam.

Michalczuk, L., Cooke, T. J., Cohen, J. D., 1992. Auxin levels at different stages of carrot somatic embryogenesis. Phytochemistry, **31**: 1097-1103.

Michalczuk, L., Ribnicky, D. M., Cooke, T. J., Cohen, J. D., 1992. Regulation of indole-3-acetic acid biosynthetic pathways in carrot cell cultures. Plant Physiol., **100**: 1346-1351.

Montague, M. J., Koppenbrink, J. W., Jaworski, E. G., 1978. Polyamine metabolism in embryogenic cells in *Daucus carota*. Plant Physiol., **62**: 430-433.

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

Nomura, K., Komamine, A., 1985. Embryogenesis from microinjected single cells in a carrot cell suspension culture. Plant Physiol., **79**: 988-991.

Nomura, K., Komamine, A., 1995. Physiological and biochemical aspects of somatic embryogenesis. In: Thorpe, T. A. (Ed.): In vitro Embryogenesis in Plants, pp. 249-266. Kluwer Academic Publishers, Dordrecht.

Norstog, K., 1970. Induction of embryo-like structures in cultured barley embryos. Dev. Biol., **23**: 665-670.

Ozias-Akins, P., Vasil, I. K., 1983. Improved efficiency and normalization of somatic embryogenesis in *Triticum aestivum* L. Protoplasma, **115**: 104-113.

Raghavan, V., 1997. Somatic embryogenesis. In: Raghavan, V. (Ed.): Molecular embryology of flowering plants, pp. 467-499. Cambridge University Press, New York.

Raghavan, V., 2000. Developmental biology of flowering plants, pp. 309-322. Springer-Verlag, New York.

Rajyalakshmi, K., Dhir, S. K., Maheshwari, N., Maheshwari, S. C., 1988. Callusing and regeneration of plantlets via somatic embryogenesis from inflorescence cultures of *Triticum aestivum* L. Plant Breed., **101**: 80-85.

Rajyalakshmi, K., Grover, A., Maheshwari, N., Tyagi, A. K., Maheshwari, S. C., 1991. High frequency regeneration of plantlets from the leaf bases via somatic embryogenesis and comparision of polypeptide profiles from morphogenic and non-morphogenic calli in wheat (*Triticum aestivum*). Physiol. Plant., **82**: 617-623.

Ryschka, S., Ryschka, U., Schulze, J., 1991. Anatomical studies on the development of somatic embryoids in wheat and barley explant. Biochem. Physiol. Pflanzen., **187**: 31-41.

Sung, Z. R., Flenberg, A., Chorneau, R., Borkird, C., Furner, I., Smith, J., Terzi, M., Lo Schiavo, F., Giuliano, G., Pitto, L., Nuti-Ronchi, V., 1984. Developmental biology of embryogeneis from carrot culture. Plant Mol. Biol. Rep., **2**: 3-14.

Sung, Z. R., Okimoto, R., 1981. Embryonic proteins in somatic embryos of carrot. Proc. Natl. Acad. Sci. U. S. A., **78**: 3683-3687.

Thakur, J. K., Tyagi, A. K., Khurana, J. P., 2001. *OsIAA1*, an *Aux/IAA* cDNA from rice, and changes in its expression as influenced by auxin and light. DNA Res., **8**: 193-203.

Thorpe, T. A., 1994. Morphogenesis and Regulation. In: Vasil, I. K. and Thorpe, T. A. (Eds.): Plant Cell and Tissue Culture, pp. 17-36. Kluwer Academic Publishers, Dordrecht/London.

Tran Thanh Van, K., 1981. Control of morphogenesis in *in vitro* cultures. Ann. Rev. Plant Physiol., **32**: 291-311.

Tran Thanh Van, K., Trinh, T. H., 1990. Organogenic differentiation. In: Bhojwani, S. S. (Ed.): Plant Tissue Culture: Applications and Limitations, pp. 34-53. Elsevier, Amsterdam.

Vasil, I. K., 1987. Developing cell and tissue culture systems for the improvement of cereal and grass crops. J. Plant Physiol., **128**: 193-218.

Vasil, I. K., Vasil, V., 1994. *in vitro* culture of cereals and grasses. In: Vasil, I. K. and Thorpe, T. A. (Eds.): Plant Cell and Tissue Culture, pp. 293-312. Kluwer Academic Publishers, Dordrecht/London.

Wenck, A. R., Conger, B. V., Trigiano, R. N., Sams, C. E., 1988. Inhibition of somatic embryogenesis in orchard grass by endogenous cytokinins. Plant Physiol., **88**: 990-992.

Wernicke, W., Milkovits, L., 1984. Developmental gradients in wheat leaves - response of leaf segments in different genotypes cultured *in vitro*. J. Plant Physiol., **115**: 49-58.

Wernicke, W., Milkovits, L., 1986. The regeneration potential of wheat shoot meristems in the presence and absence of 2,4-dichlorophenoxy acetic acid. Protoplasma, **131**: 131-141.

Wernicke, W., Grost, J., Milkovits, L., 1986. The amibiguous role of 2,4-dichlorophenoxyacetic acid in wheat tissue culture. Physiol. Plant., **68**: 597-602.

Young Soh, W., Bhojwani, S. S., 1999. Morphogenesis in plant tissue cultures. Kluwer Academic Publisher, Dordrecht.

Zamora, A. B., Scott, K. J., 1983. Callus formation and plant regeneration from wheat leaves. Plant Sci. Lett., **29**: 183-189.