LANDBAUFORSCHUNG VÖLKENRODE HEFT 4/1996 SEITE 157 - 165

Callus induction and plant regeneration from embryos in bread wheat (*Triticum Aestivum* L.)

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Introduction

Tissue culture has recently attained considerable significance for culturally induced variation, anther culture for haploids, developing transgenic plants and other biotechnological applications for plant improvement. There is considerable variation among species regarding their amenability to tissue culture. While in some virtually any part of the plant can be cultured with ease, others are more recalcitrant and only certain plant parts respond to culturing.

Wheat is one of the major food crops of the world. The green revolution in India came mainly through the improvement of wheat yields using traditional breeding methods. Further improvement may require use of new approaches together with established methods. Different parts of wheat plant such as immature embryos, mature embryos, ovary, mesocotyl, roots, immature inflorescence, immature leaves etc. have been cultured but the best starting material for obtaining embryogenic callus appears to be immature embryo (Vasil 1987, Bajaj 1990). However, availability of immature embryos is limited to only a specific season and that too for a very limited time. Mature embryos are more easily available at any time of the year but they are not as responsive as the immature embryos. Efforts have been made to improve their culturability. Success with other explants is very low. We have reviewed here various factors affecting use of embryos for tissue culture.

Factors affecting callus induction and plant regeneration

Callus induction and regeneration

Immature embryos are considered to be the best material for obtaining embryogenic calli (Eapen and Rao 1982, 1985, Maddock 1985, Butenko et al. 1986, Vasil 1987, Tuberosa et al., 1988, Subhadra and Maherchandani 1991, Omelianchuk et al. 1992). The increased efficiency of embryogenic callus formation and regeneration of plantlets was the result of using immature induced polyembryos rather than single embryos as primary explants (Erdelská and Vidovencová, 1996). Gosch-Wackerle et al. (1979) cultured whole immature garins and obtained calli which most probably originated from immature embryos. Callus initiation, growth and regeneration are significantly affected by the developmental stage of the embryos at which these are explanted. Fast growing and regenerable calli with high frequency were derived from scutellar tissues of 10-15 day old embryos (Sears and Deckard 1982, Maddock et al. 1983, Bajaj 1986, Mathias et al. 1986, He et al. 1986, 1988, Carman et al. 1988a, b) while Dahiya et al. (1995a) and Sidana et al. (1995a) could obtain plantlets from calli raised from 16-20 days old embryos. The optimum age for embryo inoculation was different for each variety and fell on one of the 16-18 days after pollination (DAP) in spring wheat and the 19-22 DAP in winter wheat (Zhang 1987). The presence of scutellum was important in culturing immature embryos (Zhang and Seilleur 1987). However, immature embryos are available only for a period during the growing season while embryos excised from mature seeds are easily available at all times. Callus could be induced from mature embryos of *T. aestivum* (Chin and Scott 1977, Eapen and Rao 1982, Lazar et al. 1983, Zhou & Lee 1983, Heyser et al. 1985, Butenko et al. 1986, Zhang and Seilleur 1987, Tuberosa et al. 1988, Shegebaev et al. 1990, Dahiya et al. 1995b and Sidana et al. 1995b). Significant varietal differences were noted in callus growth rates. In all the genotypes embryos from immature seeds proved to be better for culturing (Lazar et al. 1983). Mature scutellum did not produce embryogenic callus (Heyser et al. 1985). Zhang and Seilleur (1987), Avenido et al. (1988) reported that callus induction and plant regeneration were higher after excision of the scutellum.

Immature embryos of T.aestivum form callus mainly from scutellum and epiblast. If the embryos are cultured with the axial surface in contact with culture medium callus proliferates mainly from scutellum, but if the embryos are inverted that is with the scutellum in contact with the medium, callus proliferates from epiblast (Heyser et al. 1985, He et al. 1986). Calli from both scutellum and epiblast are embryogenic, as envisaged by their ability to form multiple plantlets (Ozias-Akins and Vasil 1983a, c, Heyser et al. 1985, He et al. 1988, 1990). Embryogenic callus induction was highest when embryos were oriented with scutellum facing upwards (Eapen and Rao 1985, Zhang 1987, D'yachuk et al. 1990) and cut in half longitudinally (Eapen and Rao 1985). Somatic embryos were induced from scutellar callus of immature zygotic embryos (Nambisan and Chopra 1992). The induction frequency of scutellum callus and production of plantlets was found to be on an average higher than that of epiblast callus (He et al. 1988) and being the highest when the scutellum was in contact with the medium (Ozias-Akins and Vasil 1983a).

Regeneration of plants from callus cultures may occur through somatic embryogenesis (Ozias-Akins and Vasil 1982, 1983, Maddock et al. 1983, Heyser et al. 1985, Mathias et al. 1986, He et al. 1986) or organogenesis (Eapen and Rao 1982, Sears and Deckard 1982, Maddock et al. 1983, Mathias et al. 1986, Bartók and Sági, 1990). While Erdelská and Vidovencová (1996) reported plant regeneration via embryogenesis, organogenesis, and by a combined process beginning as embryogensis and proceeding as organogenesis. Regeneration of plants was better from callus cultures of immature embryos. Callus induced from mature embryos too regenerated

plants but at a lower rate (Chin and Scott 1977, Gosch-Wackerle et al. 1979, Eapen and Rao 1982, Sears and Deckard 1982, Maddock et al. 1983, Heyser et al. 1985, Maddock 1985, Butenko et al. 1986, Chawla and Wenzel 1987, Lazar et al. 1983, 1987, Scowcroft 1987, Barabonova et al. 1988, Hashim et al. 1988, He et al. 1988, Sidorova et al. 1988, Tuberosa et al. 1988, Bartók and Sági 1990, Lhotova and Kucera 1990, Mohmand and Nabors 1990, Redway et al. 1990, Wang and Nguyen 1990, Whelan 1990, Cheng et al. 1992, Guenzi et al. 1992, Obertnur et al. 1993, Dahiya et al. 1995a, b). Mackinnan (1986), however, has reported similar regeneration frequency - above 90 % - from mature as well as immature embryos. Butenko et al. (1986) and Tuberosa et al. (1988) observed no regeneration from callus induced from mature embryos.

Effect of light on callus induction and regeneration

The effect of light on callus induction appears to vary with genotypes as well as the growth regulators in the culture medium. Increased callus induction frequency in the dark was reported by He et al. (1986), Dahiya et al. (1995a) while Maddock et al. (1983) and Lazar et al. (1983) observed no differences in frequency of callus induction under different light intensity. Carman et al. (1988b) observed significantly less embryoid formation when cultures were incubated under diffused light (16h photoperiod). However, Papenfuss and Carman (1987) found that this effect depended on the type of auxin used in the medium. The positive effects of dicamba on callus growth and shoot formation in the dark were significantly reduced when calli were exposed to a 16h photoperiod. Callus raised in dark showed a higher regeneration potential in cvs HD 2009, WH 147 and Kharchia 375 except in cv C 591 where the callus induced in diffused light showed better regeneration potential (Dahiya et al. 1995a). Regeneration potential of callus cultures of cv 306 induced in dark and under diffused light was almost identical (Dahiya et al. unpublished). The highest frequency of white embryogenic tissue formation and the greatest number of normal embryoids were obtained in the dark (Ozias-Akins and Vasil 1983c).

Culture media

Several standard media and their modifications have been successfully used to induce callusing and plantlet regeneration. Murashige and Skoog (1962) medium, LS medium (Linsmaier and Skoog 1965), Chin and Scott medium (Chin and Scott 1977). T-medium (Gosch-Wackerle et al. 1979), potato medium (Lazar et al. 1983) have been used successfully. Various modified MS media such as cations of half concentration (Ahloowalia 1982), microelements decreased by 50 % (He et al. 1986), double strength inorganic salts (Carman et al. 1988a, b), two fold concentrations of MS vitamins and other organic additives (Ozias-Akine and Vasil 1983a) have all been successfully used. Tuberosa et al. (1988) did not obtain totipotent callus on Gamborg's B5 medium but Pershina et al. (1988) got regeneration on the same medium.

Itu and Abe (1990) reported that plant regeneration on MS medium was 6 to 8 times higher than on B_5 and N_6 medium, Breiman et al. (1987) reported that 85 % of calli regenerated plants on VKM medium, Gaponenko et al. (1985) found Linsmaier and Skoog medium RK 64 superior over B5 and Green and Phillips medium in inducing morphogenetic callus and plant regeneration. Among organic additives the effects of coconut milk (CM), casein hydrolysate (CH), yeast extract on embryogenic callus induction have been investigated. CM promoted initiation, growth and regeneration of immature embryo callus and was more effective than CH which resulted in slow growing calli with reduced regeneration capacity (Ozias-Akins and Vasil 1982, 1983b, Maddock et al. 1983). Liu et al. (1992) observed that the addition of CH at 4 g/l improved the morphological appearance and growth rate of callus and its regeneration ability. The optimum concentration of sucrose is 2 %-3 % (Qzias-Akins and Vasil 1983b, He et al. 1986). Glucose was found to be as effective as sucrose in supporting the growth of immature embryo callus (Qzias-Akins and Vasil 1982). Addition of 3 % mannitol or 5 % PEG to basal medium caused increased embryogenesis. Sorbitol too promoted the induction of compact callus and embryo like structures (Ryschka et al. 1991).

Effect of growth regulators and adjuvants
Callus induction

Embryogenic callus develops white, round, compact organised mass with green patches (Bajaj, 1986). Embryogenic callus cultures can be most readily obtained from immature embryos by using 2,4-dichloro-phenoxyacetic acid (2,4-D) as a sole growth regulator (Sears and Deckard 1982, Maddock et al. 1983, Bajaj 1986, Mathias et al. 1986, He et al. 1988, Sidorova et al. 1988, Dahiya et al. 1995a, Sidana et al. 1995a, Fennell et al., 1986). Optimum concentration of 2,4-D for callus growth and for the inhibition of precocious germination was found to be 2mg/l by Ozias-Akins and Vasil (1983c) and 1 mg/l by Maddock et al. (1983). No callus was induced at low concentration of 2-4,D or its absence (Ozias-Akins and Vasil, 1982, Maddock et al., 1983, He et al., 1986). At higher concentration (2.5 or 5 mg/l) though callus growth was more vigorous, the shoot forming efficiency was reduced. Heyser et al. (1985) found no correlation between the 2,4-D concentration and embryogenic callus initiation from immature embryos but frequency of callus formation was higher from mature embryos at higher concentration of 2,4-D (20 mg/l). Indole acetic acid (IAA), Naphthalene acetic acid (NAA), 2,4,5-trichlorophenoxy acetic acid (2,4,5-T), 3,6, dichloro-2-benzoic acid (Dicambe), 2,4,5-tricobrophenoxyroponic acid (2,4,5,C13 POP), 3,5,6-trichloro-picolic acid (picloram) are among other synthetic auxins which have also been used. Eapen and Rao (1983) obtained best callus growth with 2,4,5-Cl3 POP, Lazar et al. (1983) obtained slower growth of immature embryo callus from cv. Oasis on 2,4-D than on 2,4,5-T or picloram. Papenfuss and Carman (1987) got significantly better callus proliferation on dicamba than on 2,4-D. Dicamba as compared to 2,4-D redu-

ced precocious germination of cultured embryos and increased the number of shoots, however plantlets obtained showed less vigour and were difficult to establish in soil. Dahiya et al. (1995b) tested different concentrations of 2,4-D (3,5,7 mg/l) and 2,4,5-T (3,5,7 mg/l) on mature embryos and found that embryos of different varieties exhibited specific response to growth regulator and concentration. 2,4,5-T was found to be good for callus induction in cvs. HD 2009 and C591 but not much effective for C306, WH147 and Kharchia 375 genotypes which gave better response to 2,4-D while NAA was not at all effective. In callus induction from mature embryos 2,4,5-T was more effective than 2,4-D. Addition of 2,4,-D (2.0 mg/l), NAA (0.5 mg/l) and casein hydrolysate (0.2 mg/l) to MS medium highly improved the callusing response of mature embryos (Dahiya et al. 1995b). Zhou and Lee (1983) studied mature embryos of cvs. Chinese Spring and Frederick on 13 different auxins in the presence of 2-isopentyladenine (2-ip) and found picloram to be more effective than 2,4-D. Chinese Spring gave better response on dicamba and 2-methyl-4-chlorophenoxy acetic acid (MCPA) than on 2,4-D. NAA (2 mg/l) and IAA (2 mg/l) failed to induce callus (Ozias-Akins and Vasil 1982).

Reports regarding use of cytokinins in the culture media are often conflicting. Significant improvement in callus formation (Lazar et al. 1983), reduction in induction of callus (Ahlowalia 1982) and even inhibition of callus formation (Butenko et al. 1986) have been reported on medium containing kinetin. Zeatin promoted callus growth but reduced callus initiation (Ahlowalia 1982) while kinetin sometimes caused necrosis of callus especially at higher concentration (Yorkova et al. 1981). Benzylaminopurine (BAP) enhanced the proliferation of callus (Ozias-Akins and Vasil 1983) while kinetin had no effect (He et al. 1986).

Ozias-Akins and Vasil (1982) found that abscisic acid (ABA) at concentration of 100 µg/l or less was not inhibitory to the growth of callus. Meddock et al. (1983) could obtain embryogenic calli from large and more mature embryos (up to 25 days post anthesis) on medium containing both 2,4-D and ABA. Strong inhibition of precocious germination of cultured embryos at 0.1 mg/l concentration of ABA was observed (Morris et al. 1986) but this concentration had little effect on induction of embryogenic callus. Qureshi et al. (1989) reported that ABA and its analogues promoted embryogenic callus induction of cultured late stage embryos. Low concentrations of ABA (0.1 and 1.0 mg/l) increased embryogenesis (Brown et al. 1989). Germination of embryos excised from mature seeds also decreased with ABA (0.1, 1.0, 2.0 mg/l) and NAA. Lower concentrations of ABA promoted callus induction while with higher concentration although almost complete suppression of germination occurred, callus induction was also inhibited (Dahiya et al. 1995b). Thus ABA provides a partial solution to prevent germination and at the same time, maintaining the callus induction response of the tissue.

Plant regeneration

Different combinations of auxins and cytokinins have been found effective for inducing plant regeneration by different workers (Tabulated below.) Gibberellic acid 1 mg/l enhanced root formation but did not affect shoot regeneration (Ozias-Akins and Vasil 1982) while NAA (0.5 mg/l) and Kinetin (1.0 mg/l) induced rooting only.

	Growth regulator in the medium	Reference
1	Hormone free	Eapen and Rao 1982, 1985, He et al. 1986, Bapat et al. 1988, Bartok and Sagi 1990, Rakhimbeav and Kushnarenko 1992, Kendall et al.1993, Dahiya et al. unpublished.
2	Low concentration of auxin	Sears and Deckard 1982, Ozias-Akins & Vasil 1983b, Galiba et al. 1986, Mathias et al. 1986, Higgins and Mathias 1987, Dahiya et al. 1995b.
3	IAA + BA	Barabanova et al.1988, Mohamand and Nabors 1990, Dahiya et al.1995a, Bohorova et al. 1995, Fennell et al. 1996.
4	NAA + Kinetin	Dahiya et al. unpublished.
5	ABA	Qureshi et al. 1989.
6	Kinetin	Fekete and Pauk 1989.
7	IAA + Kinetin	Bajaj 1986.
8	2,4-D or dicamba + Kinetin	Papenfuss and Carman 1987.
9	IAA + Zeatin	Breiman et al. 1987, Felsenburg et al. 1987.
10	BA + NAA	Sidorva et al 1988, Dahiya et al. unpublished.
11	BA + Zeatin or Kinetin	Morozova 1988.
12	Coconut milk + Cytokinins	Maddock et al. 1983.

It appears that the differentiation of callus may be more rapid on hormone free medium but a large number of plantlets can be obtained on auxin containing medium (Bapat et al. 1988). The presence of growth regulators was essential for obtaining plantlets from older cultures (Chawla and Wenzel 1987). However, if an auxin containing medium is used as differentiating medium, an additional hormone free medium is sometimes beneficial to promote the growth of roots (Sears and Deckard 1982). Callus induction medium and genotype were found to affect the regeneration frequency. Higher number of plantlets were obtained from callus of cv. HD 2009 when the induction medium was supplemented with 2,4,5-T (5.0 mg/l) (Dahiya et al. 1995a). Zhang and Seilleur (1987) also obtained best results with a medium supplemented with 2,4,5-T.

Kato et al. (1991) treated detached ears with 2,4-D (2 mg/l) for 3 min. at different days after anthesis. After ripening embryos were cultured on MS medium with 2,4-D and BA. The regeneration capacity of calli decreased with addition of BA into the subculture medium and increased by pre-treatment with 2,4-D in later stages of ear development. Regeneration ability of calli decreased with an increase in the concentration of deoxynivalenol (Vomi-

toxin) in the medium (Lui et al. 1991, Menke and Zimmy 1991) while shoot regeneration was effectively promoted by higher concentration of copper ion (5 to 1000 times) than in the original MS medium (Purnhauser 1991) or added acetone (1.5-3.0 %) (Vnuchkove et al. 1993). Treatment of immature caryopses with N-methyl-N-nitrosourea (0.005 % for 15h) before culture of the embryos inhibited embryogenic callus development and regenerative ability though this inhibiting effect was variety specific (Bannikova et al. 1990).

Addition of NaCl suppressed the differentiation of shoot apices, resulting in development of more typical somatic embryoids. The frequency of somatic embryoid formation was maximum in Chinese Spring, Arthur and GK kineso while callus differentiation in Capelle Desproez declined at 40 mM NaCl and KCl while same concentration of Licl was detrimental for all the cultivars. All the salts resulted in inhibition of plantlet formation (Galiba and Yamada 1988). High frequency of white callus or embryoids was observed with high concentrations of KH_2PO_4, $MgSo_4$ and $CaCl_2$ (cf. Bajaj, 1990).

Inclusion of $AgNo_3$ in the medium promoted shoot regeneration of wheat calli (Purnhausar et al. 1987, Inagaki and Tahir 1990). In an attempt to determine whether inexpensive starches and various agars could replace Ficoll and Bacto-Agar it was observed by Simonson and Baenziah (1992) that with wheat starch the frequency of green plants regenerated increased on an average of four fold.

Omission of micro elements like iron and manganese resulted in marked decrease in yield of embryogenic callus and poor shoot formation from embryogenic callus while omission of boron, copper, cobalt, iodine and molybdenum had little effect (He et al. 1991) and among the salts NH_4NO_3 was essential for the proliferation of embryogenic callus (He et al. 1989). Addition of L-proline reduced regeneration capability in calli but prolonged callus totipotency (Tuberosa et al. 1988). Low concentration of µ-lysine (20-100 mg/l) stimulated callus growth and the formation of stem buds (Yurkova and Bileka 1987).

Subculturing and retention of regenerating capacity

Immature embryos are available during the months of February and March and plantlets obtained from calli can successfully be transferred to soil in November and December. Cultures developed during February, March (normal rabi season in India) have to be maintained for 5-6 months till the following rabi season so that the plants may be regenerated at suitable time for transfer to soil in green house. Regeneration potential of embryogenic callus can be maintained for a prolonged period by regular transfer of the callus to fresh medium, though regeneration frequency decreases markedly with increasing age of the callus (Ozias-Akins and Vasil 1982, Sears and Deckard 1982, He et al. 1986, Chawla and Wenzel 1987, Morozova 1988, Wang and Nguyen 1990, Dahiya et al. 1995a, Fennell et al. 1996). Dahiya et al. (1995a) observed a decline in regeneration potential from 58.8 %, 44.5 %, 60.3 %, 31 %, 44.4 % to 26.3 %, 40 %, 25 %, 27.8 %, 32.6 % for five wheat genotypes namely HD 2009, C 591, Kh 375, WH 147 and C 306 after 5-6 subcultures when the callus induction medium had 2,4-D (5.0 mg/l). However, a sharp enhancement in the regeneration frequency from 61.5 to 89.6 % of cv. HD 2009 was observed when calli after putting for some days on medium supplemented with 2,4,5-T (1.0 mg/l) were transferred to auxin free medium. This regeneration frequency was comparable to 96 % which was obtained after first subculture when the induction medium was 2,4,5-T. Fennell et al. (1996) found E3 medium (MS medium containing 2,4-D) to be the best for maintaining regeneration potential after four subcultures.

The shoot regeneration ability of a number of genotypes can be maintained for over one year, however, selection of embryogenic portion of the callus and cutting off of the root forming tissue is often required during subculturing the callus. Yang et al. (1991) reported that embryogenic potential could be retained by continuous selection of embryogenic clumps at subculture. Further enhancement of the differentiation frequency of calli was observed when $AgNO_3$ was added to subculture medium.

There is also genetic variation for long term maintenance of regeneration potential which is important to plant breeders because somaclonal variation, a source of potentially useful genetic variation (Hashim et al. 1988, 1990) increased with time in culture.

Genetic control of tissue culture response

It has been stated above that wheat genotypes differ with respect to their efficiency of callus induction, callus growth rate and regeneration capacity of calli (Eapen and Rao 1982, Sears and Deckard 1982, Lazar et al. 1983, Maddock et al. 1983, Gaponenko et al. 1985, Mathias and Simpson 1986, Lazar et al. 1987, Zhang and Seilleur 1987, Barabanova et al. 1988, Sidorova et al. 1988, Tuberosa et al. 1988, Cai et al. 1989, D'Yachuk et al. 1990, Itu and Abe 1990, Lhotova and Kucera 1990, Redway et al. 1990, Subhadra and Maherchandani 1990, Redway et al. 1990, Chowdhary et al. 1991, Felfoldi and Purnhauser 1992, Dahiya et al. 1995a, b, Fennell et al. 1996). Out of 39 winter wheat lines plants were regenerated from 18 lines and the line ND 7532 had a higher percent of callus induction and regeneration (Sears and Deckard 1982). The available literature reveals that frequency of regeneration ranged from 0-96 %. However, Vasil(1987) considered growing conditions to be more important than genotype. No correlation between callus induction and plant regeneration from callus has been observed (Barabanova et al. 1988).

In vitro response is significantly influenced by genotype and growth regulator interaction (Zhou and Lee 1983, Papenfuss and Carman 1987). A growth regulator which promotes maximal response from one genotype may not be as effective, or induce a uniform response, among a range of genotypes. Similarly the effect of organic additives like CM on the formation of shoots may be positive, neutral or negative depending on the genotype (Mathias and Simpson 1986). Besides genotype physiological condition of the embryo (expressed as sum of mean daily temperatures higher than 15° C from pollination to culture of embryo on the medium) is another important factor affecting callus formation as reported by Lyntenko et al. (1992).

Genetic control of tissue culture response (TCR) in wheat has been analyzed by available aneuploid, chromosome substitution, isogenic and alloplasmic lines. No differences for callus induction were observed between Cheyenne (donor) and Chinese Spring (recipient) wheat varieties and the members of substitution series (Galiba et al. 1986), however, callus regeneration was reduced by chromosome substitution as compared to Chinese Spring. They concluded that genes controlling regeneration ability are primarily located on 1D, 7B and 7D chromosomes. There was no difference in the frequency of callus induction however, 4B chromosome substitution of variety Cappelle Desprez into Chinese Spring and several other 4B chromosome substitutions resulted in significant increase in shoot regeneration from callus (Mathias and Fukui 1986, Higgins and Mathias 1987).

Sidana et al. (1995a) cultured immature embryos of the parents (C591 donor, CS recipient) and substitution lines on MS medium supplemented with 2,4-D or 2,4,5-T (5 mg/l). Both the auxins were found to be equally efficient for callus induction as callusing frequency from most of the substitution lines was uniformly high. They observed considerable differences in shoot regeneration ability of the parents and substitution lines. 4A and 4B substitutions showed better morphogenetic potential from callus raised on 2,4,5-T where as 5B and 6B lines did not regenerate plants. 3D, 5B and 7B chromosome substitutions reduced shoot regeneration ability in both the cases. Callusing frequency of mature embryos ranged from 20.3 to 93 % among the substitution lines. 2B, 3D, 6A, 6B and 7B chromosome substitutions exhibited enhanced morphogenesis. Parents had lower regeneration percentage as compared to most of the substitution lines (Sidana et al. 1995b). Thus different chromosomes seem to control shoot regeneration ability which is possibly determined by a polygenic system.

Felsenberg et al. (1987) by utilizing ditelosomics and tetrasomics and nullisomic-tetrasomics reported 2BS to be very important for differentiation of shoots and 6BL important in callus growth. Kaleikau et al. (1989) using group 2 ditelosomics and nullisomics-tetrasomics and Chinese Spring wheat have shown the involvement of group 2 chromosomes. They suggested that a major TCR gene is located on 2 DL and that 2AL and 2BS possess minor TCR genes while a major regulatory gene controlling the expression of TCR genes may be located on chromosome 2 BL. Lazar et al. (1987) reported that regeneration from callus cultures was enhanced when addition lines contained rye chromosomes 6 and 7.

In other studies conducted by Amer et al. (1992) it was reported that semi-dwarfing gene Rht 8 had only a minor effect but the day length sensitive allele (ppd 1) determined a major increase in callus growth and regeneration ability from immature embryo calli. However, there seems to exist genetical factors for TCR on the homoeologous group 2 chromosomes which may be closely linked to Ppd loci (Amer et al. 1996). Omelianchuk et al. (1992) from their studies on isogenic lines of wheat cv. Novosibirskaya 67 carrying reduced height and leaf pubescence character have suggested that the leaf pubescence gene of ANK-7A or a gene closely linked to it are likely to play an important role in the control of plant regeneration in vitro. ANK-7A produced calli regenerating plants while Novosibirskaya 67 and K-26011 (donor of leaf pubescence for ANK 7A) were completely incapable of plant regeneration showing thereby that the expression of these genes is influenced by genomic background.

Conclusions and Summary

Plant regeneration through callus cultures obtained using immature embryos in interspecific/intergeneric (Ushiyame et al. 1991, Chen et al. 1992, Girko et al. 1992) and mature embryos in intervarietal crosses (Girko and Voloshehuk, 1991) has far reaching implication in wheat improvement programmes. This is rather pertinent when wheat breeders are confronted with gene flow barriers or limitations of time and space. The success of plant regeneration through embryo cultures is apparently influenced by culture medium and manipulateable genetic factors which can be controlled and environmental factors which can not be controlled. Fortunately a strong inherent interaction among these factors exists which warrants for defining effective embryo culture protocols for accelerating the pace of wheat improvement programme aimed at enlargement of genetic variability (Larkin et al. 1984, Bajaj 1986, Ryan and Scowcroft 1987, Bannikova et al. 1990, Mohmand and Nabors 1990, Sharma et al. 1992, Oberthur et al. 1993), enhancing gene flow from one genetic background to the other and development of wheat genotypes tolerant to abiotic and biotic stresses. This review has provided comprehensive information on plant regeneration through embryo culture and warrants a closer interaction between biotechnologists, plant physiologists, geneticists and wheat breeders.

Kallusinduktion und Pflanzenregeneration aus Brotweizenembryonen *(Triticum aestivum)*.

Der Literaturüberblick stellt anschaulich da, daß die In-vitro-Kulturtechniken in den Brotweizenzuchtprogrammen einen festen Platz eingenommen haben.

Es werden die Faktoren beschrieben, die die Kallusinduktion sowie das Wachstum aus unreifen und reifen Embryonen unterschiedlichster Herkunft beeinflussen.

Der Bericht zeigt ebenfalls deutlich, daß der Erfolg einer Pflanzenregeneration durch die Zusammensetzung des Nährbodens und verschiedener Umweltfaktoren entscheidend bestimmt wird.

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