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Title: Tissue Culture and Plant Regeneration from Immature Embryo Explants of Twenty-two Genotypes of Barley, Hordeum vulgare L.

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Abstract approved:

Immature embryo explants taken eight days after anthesis were used to establish callus cultures of spring barley. Two types of calli were observed. A soft watery callus which produced a limited number of shoots and a harder yellowish callus that gave rise to numerous green primordia and shoots. Gambory's B5 basal medium supplemented with either 2,4-D 2,4-dichlorophenoxyacetic acid) or Cl_3 POP (2,4,5-trichlorophenoxypropionic acid) was found to support the growth and formation of callus capable of shoot and root development. Among the

hormone concentrations tested, 2,4-D at 1.0 mg L^{-1} and Cl_3 POP at 5.0 mg L^{-1} gave rise to numerous callus cultures capable of plantlet regeneration.

Twenty genetically diverse genotypes were screened to determine if the methods developed were suitable for tissue culture propagation of spring barley cultivars. Regeneration of plantlets was achieved for 19 of the 20 genotypes approximately 4 months after culture initiation. Differences were detected in the ability of these genotypes to develop into vigorously growing callus cultures capable of shoot and root initiation. Specific cultivars and advanced breeding program lines have been identified for their ability to produce many shoots and several plantlets from a single embryo explant.

Evidence for heterosis in tissue culture was found for callus production, root and shoot development, and regeneration of plantlets in four spring barley crosses. A heterotic effect was detected in all F_1 tissue culture populations for plantlet regeneration and gain in fresh weight 9 weeks after culture initiation. The results clearly demonstrate that heterosis was present in F_1 tissue culture populations from spring barley crosses. The increase in numbers of plants regenerated suggests that F_1 materials are well suited for tissue culture studies directed towards the genetic improvement of cereal crops.

Tissue Culture and Plant Regeneration from Immature Embryo Explants of Twenty-two Genotypes of Barley, *Hordeum vulgare* L.

by

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GENERAL INTRODUCTION

The use of tissue culture as a tool in plant improvement is a potential application of biotechnology to agriculture. Major obstacles to the application of this technology include the need for predictable, high-frequency, long-duration regeneration of plants from tissue culture. As these procedures become more refined, factors hampering the practical application of this technology will be overcome.

Several steps must be taken to allow this biotechnology to become integrated into more traditional plant breeding programs. Two factors must be studied to achieve this goal. First, it must be determined which tissues are most likely to give rise to shoots and roots. Secondly, the discovery of a tissue culture propagation medium that will support vigorous callus growth is needed. Also the optimal balance of growth-promoting hormones must be found that will ensure the development of both shoots and roots, thus producing whole plantlets. The achievement of high-frequency regeneration of plants is a final prerequisite to the successful development of a tissue culture propagation methodology. Maintenance of cultures for several months or longer still has not been well achieved in tissue culture research with cereal crops.

One step that would bring researchers closer to the understanding of this limiting factor is the identification of genotypes that do respond vigorously in tissue culture. Specific genotypes must be

identified for their ability to develop callus, shoots, and roots rapidly. Once a number of genotypes are found that grow vigorously and develop numerous plantlets, studies can be undertaken to determine the genetic basis of successful in vitro propagation. Cultivars that respond vigorously in culture will be useful for developing a model system for the long-term regeneration of plants from tissue culture.

These studies address strategies for reaching the objective of predictable tissue culture regeneration of barley plants. Research was carried out to determine the best culture medium and optimum hormonal balance for callus, shoot, and whole plantlet development. Specific genotypes have been identified for their ability to give rapid callus growth and high yields of shoots and plantlets.

A second study was conducted to determine whether hybrid vigor influences callus growth or the regeneration frequency of barley tissue cultures. If heterosis is expressed in certain F_1 populations these materials will serve as a source for further applied tissue culture studies.

LITERATURE REVIEW

There is a particular sequence of procedures that are followed when attempting to develop tissue culture methods for a specific crop species. The literature review will discuss the step-wise progression of a series of studies that will finally lead to the growth of callus cultures and regeneration of shoots and complete plants from tissue cultures. The first decision is which organ or tissues of the plant will be most suitable for explanting. Secondly a nutrient growth media must be found that provides the balance of vitamins, minerals, sugars, and other additives to support vigorous growth. At the same time a search for the proper medium is carried out, it is also necessary to test a range of growth hormones at varying concentrations to find an optimum hormonal balance. There are three stages of development in the tissue culture process. The hormonal requirements may differ for any one of these stages of callus induction, shoot initiation, and root initiation. Once plants can be regenerated with shoots and roots it is desirable to be able to regenerate numerous plants from a single initial explant and to be able to reliably predict what the regeneration potential is for a particular genotype. It is not unusual to observe variation in the responses of genotypes of a single species when they are propagated by in vitro methods. One of the disappointing findings for culturing any of the cereal crops is the loss of ability to regenerate plants over time.

In the case of barley, Hordeum vulgare, studies were carried out by several investigators to determine which materials would be most suitable for establishing cultures. A number of factors govern an

assessment of suitability. Ease of excising the plant materials, cleanliness and contamination of the tissues, and quantity of materials available from a single source plant should be considered. Most importantly the success of achieving the final objectives of callus development and vigorous growth must be evaluated.

A number of investigations have been conducted with barley using a range of source tissues. Cheng and Smith (1975) were able to establish rapidly proliferating callus cultures from apical meristems of barley cultivars "Himalaya" and "Mari". Plants were also obtained from callus derived from shoot apices by Koblitz and Saalbach (1976). Morphogenesis of barley tissue was later reported by Weigel and Hughes (1983) using 1 cm long explants consisting of the apical meristem and adjacent stem tissue. Immature embryo explants were used by Deambrogio and Dale(1980) to study the effects of 2,4-D on plant regeneration. Mature seed embryos of barley were used in studies conducted by Kartel and Maneshina (1978) and by Bayliss and Dunn (1979). Mature embryos were also used as a source of explant materials by Dunwell(1983) in studies to examine the influence of temperature regimes on growth of mature embryos in vitro. Jelaska et al.(1984) were able to generate shoots and plantlets from barley mesocotyl explants of germinated 7 day old seedlings.

Based on studies conducted by Dale and Deambrogio (1979) reporting immature embryo explants as their best explant source, this was the choice made for the experiments conducted in this study. Immature embryos have been found to be a suitable source of explant material in other monocot species including maize (Green and Phillips 1975), oats (Heyser and Nabors 1982), rye and triticale (Eapen and Rao 1982), and

proso millet (Heyser and Nabors 1982). In their research with barley tissue cultures Dale and Deambrogio (1979) were able to discover distinct differences in the suitability of explant tissue sources for their abilities to produce callus capable of plant regeneration. Immature embryos of barley gave the higher frequencies of callus initiation and subsequent growth of callus when compared with meristem tips, leaf sheath tissue, mesocotyl region, root, and mature embryo explants.

Dale and Deambrogio (1979) described a relationship between time after anthesis and embryo length. The objective in the selection of explant materials was to elicit rapid callus establishment and growth while avoiding precocious germination of the embryo. Descriptions of the morphological staging of wheat caryopsis development from Rogers and Quatrano (1983) were followed to determine the stage at which explants would be most suitable for culture initiation. The specific stage of physiological maturity that was found to be optimal corresponds with Stage II of Rogers and Quatrano. The characteristics of these immature embryos are developmental age of 7 to 8 days after anthesis, caryopsis color a white to minty green, and embryo length of 0.5 to 1.0 mm.

Any study conducted to develop methods for culturing a new plant species or genotype must include a survey of the growth media found successful for closely related cultivars. The most universally tested growth media is one developed by Murashige and Skoog (1962) for tobacco tissue culture, commonly referred to as MS (M and S) media in the literature. It has been used by many researchers working on a wide range of cereal crops. In the case of Triticum aestivum MS media

has been used by Eapen and Rao (1982 c), Ozias-Akins and Vasil (1983), Ahloowalia (1982), and Sears and Deckard (1982). Plant regeneration studies with Durum and Emmer wheat, Triticum turgidum L. Thell ssps durum and diococcum by Eapen and Rao (1982 a) were carried out with MS media. Eapen and Rao (1982 b) also conducted studies with rye and triticale tissue culture using Murashige and Skoog's basal medium. Murashige and Skoog's medium was found to be very suitable for tissue culture development of spring wheat cultivars tested. It was not, however the most suitable medium for barley tissue cultures.

Although Murashige and Skoog's media is used in a number of barley tissue culture studies a second media has been tested. Other investigators have reported the use of B5 basal medium for barley tissue culture including Scheunert et al. (1977), Bayliss and Dunn (1979), and Dale and Deambrogio (1979). When B5 was compared to MS the B5 media gave over 40% more callus than MS in the studies conducted by Dale and Deambrogio (1979). Gamborg's media has also been used in the tissue culture of Triticum aestivum by Dudits et al. (1975) and Bhojwani and Hayward (1977). Murashige and Skoog's medium has been used more often than Gamborg's for barley tissue culture. These references include reports by Cheng and Smith (1975), Orton (1979), and Jelaska et al. (1984).

Once a tissue culture media proves to support vigorous callus growth it is necessary to develop a balance of plant growth regulators for progressive stages of plantlet development. Numerous publications and practical guides are available describing the effects of various growth hormones on tissue cultures of various crop species. Both Fossard (1976) and Gamborg and Shyluk (1981) discuss the three

categories of growth hormones that are often reported in the tissue culture literature. Gibberellins are usually not essential for culture growth, but is sometimes used to encourage shoot elongation for meristem cultures of certain plant species. Cytokinins are provided to stimulate cell division and thus to elicit callus growth. Auxins also have an effect on the initiation of cell division and callus formation. In some species auxins are used to induce root formation. The proliferation of numerous shoots often involves the manipulation of the auxin-to-cytokinin ratio in the culture medium, with high cytokinin and low auxin levels required.

Cereal crops do not show a need for gibberelins and the reported effects of cytokinin are often conflicting. A further case which must be considered is the genotype:growth hormone interaction. More specifically Bayliss and Dunn (1979) have suggested that genetic variation could explain the differing responses of cereals to auxins and cytokinins. They were not able to show the existence of an auxin:cytokinin interaction for barley cultures. Their conclusions suggest that cytokinins have a small effect on shoot formation and that this is due more to genetic variation or genotype:auxin interaction than the more common hormone ratio effects found to be important for other crop species.

The earliest reports of successful organogenesis from barley cultures were those of Cheng and Smith (1975). They developed a CI medium for callus induction, a CM medium for callus maintenance, and an SI medium for shoot induction. The CI medium contained the synthetic auxins 2,4-D , 2,4-dichlorophenoxyacetic acid; IAA, Indole-3-acetic acid; and CPA, p-chlorophenoxyacetic acid; to maintain

maximum callus growth rates. The callus maintenance medium contained IAA in amounts similar to that of the CI medium and 2iP, the cytokinin 6-(3-methyl-2-butenyl-amino) purine. The shoot inducing medium was devoid of all growth promoters.

A wide range of plant growth regulators have been tested in tissue culture investigations of the various cereal crops. A regime that is often tested is one developed by Green and Phillips (1975) for maize regeneration. They were most successful when using MS media supplemeted with 2 mg L^{-1} of 2,4-D for the stimulation of callus growth. When placed on media containing 0.25 mg L^{-1} 2,4-D small leafy structures began to appear. The differentiation of complete seedlings from these leafy structures was accomplished by transferring to media with no 2,4-D added. A similar pattern of 2,4-D addition was followed by Sears and Deckard (1982) in their tissue culture research with thirty-nine genotypes of winter wheat. Cultures were initiated with levels of 1.0 mg L^{-1} of 2,4-D. This was reduced to 0.5 mg L^{-1} for maintenance of cultures, and further reduced to 0.1 mg L^{-1} for shoot initiation. Complete plantlet regeneration was achieved when plants were placed on media free of 2,4-D.

Most barley tissue culture studies report the testing of or exclusive use of 2,4-D as an auxin source. Bayliss and Dunn (1979) reported on the factors that effect the formation of callus from embryos of barley. They found callus growth rate to be related to 2,4-D concentration. Their results indicate that increasing 2,4-D concentrations suppressed normal root and shoot development. They were able to conclude that the reduction of 2,4-D concentration would result in the differentiation of roots. Deambrogio and Dale (1980)

reached the same conclusion. They cultured immature embryos on media containing 2,4-D at concentrations of 1-3 mg L^{-1} and at a higher exposure of 4 mg L^{-1}. The lower concentration range resulted in higher frequencies of plantlet regeneration. Fewer abnormalities were reported in the progenies of regenerated plants derived from the lower treatment levels. Nabors et al. (1983) studied the problem of long-term regeneration of cereal tissue cultures. Their strategy involved decreasing the concentration of the 2 auxin supplements 2,4-D or 2,4,5-T from callus production levels to increase regeneration of shoots. These studies by Nabors et al. were carried out with pearl millet, wheat, oats, and rice cultivars. Orton (1980) initiated cultures for chromosomal studies on media supplemented with 5 mg L^{-1} 2,4-D and maintained cultures with 4 mg L^{-1} 2,4-D. Jelaske et al. (1984) found mesocotyl callus tissue could be initiated in a high percentage of cultures with higher 2,4-D or 2,4,5-T concentrations. This would suggest that higher concentrations of auxins are necessary for callus induction of explants other than embryos.

Cytokinin studies do not seem to show positive responses from one study to the next for barley tissue culture. Bayliss and Dunn (1979) examined the effects of cytokinins and found either an absence of response or an inhibition of barley callus formation. They tested four different cytokinins and no differences in activity between these growth promoting substances was found. All showed a statistically significant inhibition of callus growth which was proportional to concentration. Dale and Deambrogio (1979) concluded that the presence of cytokinin actually reduced the amount of primary callus formed from the explant materials. They added the cytokinin 2iP to MS media

supplemented with 2,4-D and found a decrease in the amount of callus growth in these cultures. Jelaska et al. (1984) transferred calli from primary cultures to media with IAA and the cytokinin BA, (6-benzylaminopurine). Lush rooting was accomplished in this case but only one culture was capable of shoot regeneration.

A common strategy pursued in tissue culture research is the testing of methods suitable for related crop species. This was done in preliminary studies leading to this thesis. Eapen and Rao working in the Plant Morphogenesis and Tissue Culture Section of the Bhabha Atomic Research Centre of India have reported positive results obtained when testing an auxin not used by other investigators. Initiation of callus from media with 2,4-D at 5 mg L^{-1} was reported for both rye and triticale. As reported by Eapen and Rao (1982 b) a second auxin referred to as Cl_3 POP or 2,4,5-Cl_3 POP (2,4,5-trichlorophenoxypropionic acid) stimulated the regeneration of shoot buds from callus tissues derived from embryos cultured on basal medium supplemented with Cl_3 POP at concentrations of 5 mg L^{-1}. Further development of shoot buds was observed when triticale cultures were transferred to media with the cytokinin zeatin and the auxin IAA. Rye regeneration was found to occur on media supplemented with zeatin and no auxin. Rooting of both rye and triticale was accomplished by transferring to media with the auxin NAA, napthalene acetic acid. In further studies of cereal tissue culture by Rao and Eapen (1982 a) with Durum and Emmer wheat, callus cultures initiated on a basal medium supplemented with 2,4,5-Cl_3 POP at 5 mg L^{-1} were observed to develop into vigorously proliferating callus. Shoot bud regeneration was found to occur on the Cl_3 POP media. The appearance of greenish

patches from which tiny shoot buds emerged was consistent from culture to culture of both durum and dioccum cultivars. Eapen and Rao(1982 b) investigated callus cultures and plantlet formation for 6 bread wheat cultivars. Among the different growth supplements that were tested Cl_3 POP induced the best callus growth. This auxin source was shown to be an effective growth regulator for induction and maintenance of wheat callus cultures.

Many studies conducted with cereal tissue culture have reported the ability to induce callus growth and subsequent organogenesis of shoots and roots. A substantial problem now remains to be overcome. Although cultures have regeneration potential initially, there tends to be a decline in totiopotency as time goes on. Totiopotency is the term used to describe the ability of a callus culture to give rise to shoots. The longer the cultures are maintained the less frequently shoot development is observed. There are two considerations in overcoming this problem. The first is simply a matter of maintaining healthy cultures by transferring frequently. Subculturing every 3-4 weeks is ideal, it is also very tedious and time-consuming. The other consideration relates to the numerous reports of two callus types appearing in cereal cultures. This seems to be found in all of the economically important cereal crops surveyed.

Nabors et al. (1983) have studied the problem of long-term, high-frequency regeneration from cereal tissue cultures. They carefully separated the two callus types termed E, embryogenic; and NE, non-embryogenic callus. These two callus types are seen to develop side-by-side in a single callus culture. In their first series of experiments E callus was shown to always produce more plants than NE

callus. As time went on E type callus became composed of partly NE callus as well as embryogenic type. This became a greater proportion of the callus composition over time. The regenerative ability of E callus itself did not decrease over a 40 week time period. Their next set of experiments were designed to improve plant regeneration media for the cereal E callus. This was generally accomplished by the usual method of decreasing the concentration of 2,4-D or other auxin supplements to increase regeneration. This strategy is very practical. The type of callus that will give the best regeneration of plants, the so-called E or embryogenic callus is separated out when subculturing. This callus is placed on media with growth promoting substances balanced to encourage the proliferation of shoots. Sears and Deckard (1982) reported this same strategy for the predictable and stable regeneration of winter wheat genotypes. They were able to maintain plant regeneration by selecting and subculturing regenerable or shoot-producing calli.

There is controversy as to how shoots arise from callus cultures. Deambrogio and Dale (1980) have provided a feasible explanation for the loss of totiopotency over time. They describe the loss of the ability to produce shoots in barley cultures over time to be due to the way in which shoot formation occurs. Plant regeneration is not a de novo process from callus, but rather from undetected shoot and root primordia carried over from the immature embryo explant . The gradual decrease in organogenic potential of the cultures as the months increase is explained as the deterioration of these organized structures over time. There are directly conflicting reports of the process of shoot induction. Eapen and Rao (1982 b) in their studies

with rye and triticale discuss shoot bud regeneration as a de novo phenomena that occurs from the surface of the calli itself. They directly state that embryonic shoot buds are not involved and that plant regeneration is not due to the existence of depressed shoot primordia that proliferate once in culture. Ahloowalia (1982) has suggested that both of these mechanisms are occurring in the regeneration of plants from wheat callus cultures. Plants from embryo culture can originate in more than one manner according to this hypothesis. Some shoots would originate from highly suppressed primordia found in the original embryo explants. Plants that are obtained from callus cultures after approximately 6 months are categorized as genuine regenerates that were obtained from callus that had differentiated into shoot-primordia and formed bipolar structures which develop into shoots and roots.

The final consideration in these tissue culture research investigations is the determination of which genotypes are most suitable for long-term and high-frequency production of regenerates. Little information is available on this subject for barley tissue culture. A variety of genotypes have been used by tissue culture investigators. Some of these more promising cultivars were used in the genotype study conducted for this thesis project. Cheng and Smith (1975) were able to regenerate plants from "Himalaya" barley meristem cultures. "Himalaya" was also used by Bayliss and Dunn (1979) for their studies of callus formation from mature embryo explants. The cultivar "Akka" was reported as the line used by Deambrogio and Dale (1980) in their studies of the effect of 2,4-D on barley cultures.

The phenomena of hybrid vigor has been well studied in this century, and a multitude of references are available in the literature reporting research approaches to the use of hybrid vigor as a breeding strategy. There are very few reports of research conducted with hybrid vigor in tissue culture populations. Salman , Sears, and Guenzi (1984) have investigated F_1 populations in their studies of the genetic control of tissue culture in winter wheat. They have concluded that heterosis for totiopotency and callus growth rate were detectable in their winter wheat cultures. They have suggested that calli derived from F_1 embryos would be a feasible source of materials to be used in further in vitro research such as selection studies. They were also able to identify a superior parent that transferred its character for tissue culture response to the progeny as a simply inherited dominant gene.

TISSUE CULTURE AND PLANT REGENERATION FROM IMMATURE EMBRYO EXPLANTS OF BARLEY, HORDEUM VULGARE

ABSTRACT

Immature embryo explants taken 8 days after anthesis were used to establish callus cultures of spring barley. Two types of calli were observed. A soft, watery callus produced a limited number of shoots and a harder, more compact, yellowish callus gave rise to numerous green primordia and shoots. Gamborg's B5 basal medium supplemented with either 2,4-D (2,4-dichlorophenoxyacetic acid) or Cl_3 POP (2,4,5-trichlorophenoxypropionic acid) was found to give good callus growth and shoot initiation. Media containing 2,4-D at 1.0 mg L^{-1} or Cl_3 POP at 5.0 mg/l produced numerous cultures that resulted in the regeneration of plants. Plantlets developed roots on basal medium with Cl_3 POP at 1.0 mg L^{-1} or on auxin-free medium. Twenty genetically diverse genotypes were screened to determine if these techniques were suitable for a wide range of spring barley cultivars. Regeneration of plantlets was obtained for 19 of the 20 genotypes approximately 4 months after culture initiation. Lines differed in their ability to develop into vigorously growing callus cultures as well as in their relative abilities to develop large numbers of shoots and regenerated plantlets.

INTRODUCTION

Reproducible regeneration of plants has recently been reported for a number of cereal crop species including wheat (Sears and Deckard, 1982; Ahloowalia, 1982; Eapen and Rao, 1982 a), rye and triticale (Eapen and Rao, 1982 b), proso millet (Heyser and Nabors, 1982 a), oats (Heyser and Nabors, 1982 b) and barley (Deambrogio and Dale, 1979; Bayliss and Dunn, 1979). A high rate of regeneration from callus cultures is a prerequisite for the use of tissue culture as a tool in agronomic crop improvement. Plants must be able to undergo regeneration at a high-frequency. Also it is desirable to enhance regeneration for longer than 6 or 9 months.

Specific differences do exist in the in vitro responses of genetically diverse genotypes. Distinctions can be made among cultivars for their callus growth responses, ability to initiate shoots and roots, and regeneration potentials. Varieties and breeding program lines have been identified for their ability to yield numerous regenerated plantlets. The identification of specific genotypes that are capable of rapid callus production and high rates of plantlet regeneration is an important step toward the application of tissue culture techniques to agriculture .

Results will be presented to substantiate the successful regeneration of a genetically diverse range of spring barley cultivars. Experiments were conducted to determine optimal explant stage of maturity, growth regulator requirements, and distinct genotype responses. Detailed explanations of culture responses at various stages of callus growth and plantlet development are provided.

As methodologies for tissue culture propagation of cereal crops become more refined, factors hampering the practical applications of this technology will be overcome.

MATERIALS AND METHODS

The plants used in each experiment were grown under greenhouse conditions. Measures were taken to avoid stress created by limiting environmental factors. Pots containing a greenhouse soil mixture of peat, leaf mulch, and soil were watered daily. A 15-15-15 fertilizer was incorporated into the soil mixture at the time of planting and a one-tenth strength Hoagland's fertilizer solution was applied one week prior to anthesis. Plants for hormone studies were kept at constant temperatures of 35 to 38 degrees C with a 14 hour day-length. Plants for genotype studies were held in a greenhouse with daytime temperatures ranging from 26 to 38 degrees C and approximately 14 hours daylight. The seed sources for all plants were acquired from breeding program seed maintained as pure lines.

Spikes were bagged a few days before anthesis. Immature spikes were collected 8 days after anthesis. The caryopses were surface-sterilized with 20-fold diluted Clorox (5.25% sodium hypochlorite) for 5 minutes and rinsed 5 times with sterile distilled water. Embryos were excised using a dissecting microscope under sterile airflow conditions. Three immature embryo explants were taken from a single spike and placed in the same petri dish.

Immature embryo explants were placed in 15 by 60 mm pre-sterilized petri dishes. Cultures were established and maintained on Gamborg's B5 basal medium with the addition of the following components (concentrations are mg L^{-1}): $NaH_2PO_4.H_2O$, 150; KNO_3, 2500; $(NH_4)_2SO_4$, 134; $MgSO_4.7H_2O$, 250; Ferric EDTA, 40; (Gamborg and Wetter, 1975) supplemented with 2% sucrose, and .7% to .8% Sigma agar-agar. The pH

of the medium was adjusted to 5.5 with either NaOH or HCl before the addition of agar.

Any embryo explants that showed signs of precocious germination were discarded. Cultures were transferred every 3 to 6 weeks. Once shoots began to develop cultures were placed in autoclaved glass test tubes or pre-sterilized baby bottle liners. All cultures were maintained in the same growth chamber. The controlled environment chamber was kept at 24 to 26 degrees C, with a 12 hour day-length.

HORMONE EXPERIMENTS. A randomized complete block design consisting of six replicates, four treatment levels and three subsamples was used. Six plants of the cultivar "Klages" were established in the greenhouse. Four randomly selected spikes were collected from each plant and labelled according to source for testing the four hormone treatment levels. The spikes were from both primary and secondary tillers.

Three immature embryo explants were taken from a single spike and placed in a petri dish supplemented with a specific hormone concentration. These three embryos were subsamples of a replicate. The hormone treatments were 2,4-D (2,4-dichlorophenoxyacetic acid) at 1.0 mg L^{-1} and Cl_3 POP (2,4,5-trichlorophenoxypropionic acid) at concentrations of 1.0, 2.5, and 5.0 mg L^{-1}. Measurements were taken for callus gain in fresh weight, appearance of green primordia, shoot and root initiation, and plantlet regeneration.

GENOTYPE EXPERIMENTS. A randomized complete block design consisting of five blocks and 20 genotypes was used. One spike was randomly selected from each of the 100 plants in the investigation. Three immature embryo explants were excised from the spike and placed in a

culture dish. These three embryos were subsamples of the blocks.

Cultures of 20 genetically diverse genotypes were initiated on Gamborg's B5 medium supplemented with 1.0 mg L^{-1} 2,4-D. Cultures were maintained on this same medium from initiation of callus through plantlet regeneration stages. Regenerated plantlets were transferred to hormone-free media to stimulate root development. Successful growth and regeneration evaluations were based on measurements of callus fresh weight gain, shoot and root initiation, and plantlet regeneration.

RESULTS

No differences were detected in callus growth, whether the scutellum was placed in contact with the medium or facing up. There were obvious differences in the response of embryos taken at various developmental stages. Embryos taken 7 to 8 days after anthesis were most easily established into vigorously growing calli. This is the equivalent of stage II in wheat caryopsis development as described by Rogers and Quatrano (1983), when the caryopsis is at the milky stage. Not only were the number of days after anthesis important, but the size of the explant had a distinct effect on the success of establishing cultures. Embryo lengths of 0.5 to 1.0 mm were best for eliciting rapid callus development while avoiding the precocious germination of the embryo.

HORMONAL RESPONSES. All auxin concentrations tested were suitable for the initiation of vigorously growing callus cultures. Significant differences existed in the effect of the growth regulators on callus fresh weight gains at both 4 weeks and 9 weeks after explants were established. Supplements of either 2,4-D at 1.0 mg L^{-1} or Cl_3 POP at 1.0 mg L^{-1} caused the greatest callus production by explanted embryos.

Table 1. Hormone treatment means for fresh weight of callus cultures 4 weeks after initiation for the cultivar Klages.

TREATMENT ($mg\ L^{-1}$)	FRESH WEIGHT (grams)
2,4-D 1.0	.289 a
Cl_3 POP 1.0	.228 a
Cl_3 POP 2.5	.149 b
Cl_3 POP 5.0	.118 b

1) Mean of six replications.

2) Means with a letter in common are not significantly different.

$LSD_{.05}$ for comparison among these means = 0.078.

Table 2. Hormone treatment means for fresh weight of callus cultures 9 weeks after initiation for the cultivar Klages.

TREATMENT ($mg\ L^{-1}$)	FRESH WEIGHT (grams)
Cl_3 POP 1.0	.956 a
CL_3 POP 2.5	.724 b
2,4-D 1.0	.633 bc
CL_3 POP 5.0	.483 c

1) Mean of six replications.

2) Means with a letter in common are not significantly different.

$LSD_{.05}$ for comparison among these means = 0.182.

Green primordia began to appear 4 to 6 weeks after embryo explants were established. The addition of Cl_3 POP at 1.0 mg L^{-1} often resulted in the formation of green roots from these primordia rather than the more desirable initiation of shoots. Hormone treatments did not differ in their effect on green primordia initiation. The ranking of hormone treatment effects changed from evaluations at 9 weeks compared with those measured at 12 weeks. At 9 weeks, Cl_3 POP at 1.0 mg L^{-1} gave the greatest number of green primordia. The measurements recorded at 12 weeks show 2,4-D to be the better treatment for green primordia initiation. Those cultures exposed to Cl_3 POP at 5.0 mg L^{-1} consistently ranked lowest for formation of green spots.

The optimum auxin treatments for induction of shoot-producing callus were Cl_3 POP at 2.5 and 5.0 mg L^{-1}. These growth regulator treatments gave produced the largest numbers of shoots. Although these results were not statistically different at the .05 probability level, the greatest mean value for shoot emergence at 9 weeks were for cultures with Cl_3 POP levels of 2.5 mg L^{-1}, and at 12 weeks for Cl_3 POP levels of 5.0mg L^{-1}

Plantlets were regenerated approximately 12 to 16 weeks after initial embryo excision. Plantlets developed for all four of the hormonal levels tested. Analysis of data collected 6 months after culture establishment proved that significant differences existed for the regenerative capacity. The greatest number of plants were regenerated for those cultures with Cl_3 POP concentrations of 5.0 mg L^{-1}.

Table 3. Hormone treatment means for regeneration of plants 6 months after initiation of cultures for the cultivar Klages.

TREATMENT (mg L^{-1})	NUMBER OF PLANTS
Cl_3 POP 5.0	1.6 a
Cl_3 POP 2.5	1.1 a
2,4-D 1.0	0.6 b
Cl_3 POP 1.0	0.1 c

1) Mean of six replications.

2) Means with a letter in common are not significantly different at .05 level of probability. $LSD_{.05}$ for comparison among these means = 0.42.

GENOTYPE RESPONSES. Callus was formed within the first 3 weeks. Genotypes ranged in their relative callus-forming ability scored 6 weeks after culture initiation. The cultivar Gus was found to have twice the weight gain compared to the mean of all twenty genotypes. The cultivars Himalaya, Klages, Morex, and Triumph ranked in the top 25% for the barley populations evaluated.

Table 4. Mean value for gain in fresh weight of callus cultures 6 weeks after initiation for twenty cultivars of spring barley.

GENOTYPE	FRESH WT (grams)
Gus	.645 a
Klages	.452 b
Himalaya	.450 b
Triumph	.410 bc
Morex	.403 bcd
Apam Dwarf	.359 b-e
Or 7334-3	.341 b-e
Minn 66-102	.299 b-f
Diamant	.288 c-f
OSB 763390	.281 c-f
Multum	.235 d-g
Akka	.227 e-h
ORSS-2	.224 e-h
Karl	.205 e-h
Advance	.134 fgh
Short Wocus	.134 fgh
Benton	.116 gh
C2-79-198	.103 gh
Steptoe	.088 gh
Mex 79132-Hk	.071 h

1) Mean of five replications.
2) Means with a letter in common are not significantl different at the .05 probability level. $LSD_{.05}$ for comparison among these treatment means = 0.159.

Four weeks after explanting the earliest formation of green spots was noted. Shoots and occasionally roots arose from these green primordia. Significant differences in shoot initiation from the green primordia were observed. Cultivars and advanced breeding lines have been selected for their prolific shoot development. Apam Dwarf and Advance were two cultivars that produced an average of more than five shoots from a single culture. Multum and the breeding line OSB 763390 from Oregon State University both yielded an average of four shoots per initial explant.

Table 5. Treatment means for shoot development 12 weeks after culture initiation for 20 spring barley cultivars.

GENOTYPE	NUMBER OF SHOOTS				
Apam Dwarf	6.1	a	Minn 66-102	2.1	c-f
Advance	5.3	ab	Mex 79132-Hk	1.6	c-f
OSB 763390	4.5	abc	Diamant	1.6	c-f
Multum	4.2	a-d	Akka	1.3	def
Karl	3.7	a-d	Benton	1.1	def
Gus	3.2	a-e	Triumph	1.1	def
ORSS-2	2.9	b-f	Morex	0.3	ef
OR 7334-3	2.9	b-f	Short Wocus	0.3	ef
C2-79-198	2.9	b-f	Steptoe	0.1	ef
Klages	2.6	b-f	Himalaya	0.0	f

1) Mean of five replications.
2) Means with a letter in common are not significantly different. $LSD_{.05}$ for comparison among these means = 3.1.

Regeneration of plants was achieved for 19 of the 20 genotypes of spring barley investigated. Himalaya barley was second in ranking for callus weight gain but produced no plantlets. Genotypes demonstrated differences in their capability to regenerate plants with both shoots and roots. Fifty percent of the genotypes surveyed gave rise to an average of one regenerated plant from each initial embryo explant. The cultivar Multum produced two shoots per explant, the greatest mean regeneration value found in this study.

Table 6. Treatment means for regeneration of plants for 20 spring barley cultivars.

GENOTYPE	NUMBER OF PLANTS	GENOTYPE	NUMBER OF PLANTS
Multum	2.1 a	Akka	0.9 b-g
Apam Dwarf	1.8 ab	Klages	0.9 b-g
C2-79-198	1.7 abc	Benton	0.8 b-g
Karl	1.7 abc	Diamant	0.8 b-g
Mex 79132-Hk	1.7 abc	Steptoe	0.7 c-g
OR 7334-3	1.3 a-d	Minn 66-102	0.5 d-g
Advance	1.2 a-e	Triumph	0.3 d-g
OSB 763390	1.1 a-f	Short Wocus	0.3 d-g
ORSS-2	1.1 a-f	Morex	0.1 fg
Gus	0.9 b-g	Himalaya	0.0 g

1) Mean value of five replications.
2) Means with a letter in common are not significantly different at the .05 probability level. $LSD_{.05}$ for comparison among these means = 1.00.

Simple correlation coefficients were calculated for four tissue culture traits. Correlations between weight gain of 6 week-old callus cultures and shoot initiation were low. Root initiation was positively correlated at the .10 probability level to weight gain at 6 weeks. A significant positive relationship was found for root initiation with shoot initiation at the .01 level of probability. The relationship for fresh weight gain at 6 weeks and plantlet regeneration 6 months after initial establishment was found to be negative at the .10 probability level.

Table 7. Simple correlation coefficients between four tissue culture traits for 20 spring barley genotypes.

	Shoots (12 wks)	Roots (12 wks)	Regeneration (6 mos)
Weight gain (6 wks)	0.06	0.38	-0.37
Shoots (12 wks)		0.56**	0.73**
Roots (12 wks)			0.35

**: Significant at the 0.01 level of probability
1) Each correlation coefficient is based on twenty means.

DISCUSSION

The physiological maturity of immature embryo explants was found to be of the utmost importance in attaining vigorous callus growth and avoiding precocious germination of the explant. A specific size and number of days post anthesis were identified as being optimal for barley explant materials. Dale and Deambrogio (1979) described the relationship between time after pollination and mean embryo length. They concluded that immature embryos in the size range of 0.7 to 1.4 mm, corresponding to 10 to 13 days after pollination were best suited for obtaining vigorous callus induction and higher callus yields. Embryos excised 10 to 13 days after anthesis from plants grown under greenhouse conditions in Corvallis, Oregon were found to be much larger than predicted by Dale and Deambrogio. The result was precocious germination of the explant. Under local conditions a narrower window of 7 to 8 days after anthesis was the optimal stage of embryo maturity. The excised immature embryo explants in these studies were all 0.5 to 1.0 mm in length. Callus growth from these embryos was vigorous, and rapid gain in fresh weight was measured.

Two distinct callus types were observed. A soft, watery, translucent callus, and a second yellowish, more friable callus were seen. These two types of calli often appeared side-by-side in cultures derived from a single explant. More than two discrete classes of callus types probably exist in these cultures. Orton (1979) has characterized five morphologically distinct categories of callus in barley exhibiting a range of regeneration potentials. The callus can be described according to Orton's system as being of type A

or type E.

HORMONE EXPERIMENT Barley cultures initiated on media supplemented with 2,4-D consistently formed callus with shoot development capability. Shoot initiation was stimulated by both of the auxin sources tested. Rooting was best accomplished by lowering the hormone concentration to 1.0 mg L^{-1} Cl_3 POP or by removing all growth-promoting supplements.

Plant regeneration was obtained for all growth hormone treatments. The greatest capacity for regeneration was observed from cultures with Cl_3 POP at concentrations of 2.5 or 5.0 mg L^{-1} for the single cultivar evaluated. A similar response has been reported for the effect of Cl_3 POP on other cereal crop tissue cultures. Shoot bud regeneration was observed in primary cultures of rye and triticale after 3 to 4 weeks when Cl_3 POP at 5.0 mg L^{-1} was used as a growth promoter (Eapen and Rao 1982 a). Eapen and Rao (1982 b) also tested the effects of Cl_3 POP on callus cultures of"Durum" and "Emmer" wheat. Embryos initiated on Murashige and Skoog's medium supplemented with 5.0 mg L^{-1} Cl_3 POP were reported to demonstrate a superior callusing response. Shoot buds with leaves emerged from cultures with Cl_3 POP added as a growth promoting substance. Further studies of barley shoot regeneration utilizing this auxin source are in order.

GENOTYPE EXPERIMENTS Cultivars can be selected for superior growth and development in tissue culture. Genotypes differed in their callus formation abilities. Genotypes that are capable of rapid and vigorous callus initiation have been identified. These cultivars and advanced breeding lines will be valuable sources of materials for selection studies involving callus screening techniques.

Only one genotype surveyed formed callus cultures that did not produce shoots. The cultures derived from the cultivar "Himalaya" produced a single callus type that was watery and translucent. Some green spots or sectors did appear within 2 of of the 15 Himalaya cultures scored. However, no shoots or roots formed from any of these calli. Regeneration of Himalaya barley has been reported previously (Cheng and Smith, 1975). The differing results of this investigation are due to the source of initial explant materials. Apical meristems were the source of materials for culture initiation in the research of Cheng and Smith. This would suggest the importance of selecting an explant source that produces callus capable of regeneration.

The other 19 genotypes were able to undergo shoot and root development. Fifty percent of the genotypes evaluated were capable of producing at least one plant per initial explant. The regeneration of plants for all but one of the genotypes is a noteworthy finding. The methods tested proved to be successful for the tissue culture regeneration of cultivars from genetically diverse backgrounds. A wide variety of barley cultivars not yet tested are likely to respond to these in vitro propagation methods.

A number of chlorophyll deficient mutants were recovered. The cultivars Akka, Klages, and Triumph each yielded between five and ten chlorophyll mutants. This is not an uncommon phenomena in barley tissue culture populations (Dale and Deambrogio, 1979 and Saalbach and Koblitz, 1977).

There was a large degree of variability among observations. A range of developmental responses was often observed among the three subsamples of a single replicate. Within any single rep, often only

one or two calli generated numerous shoots. A predictable proportion of initial embryo explants would not be expected to demonstrate the capacity for regeneration. This variability must be anticipated when designing tissue culture experiments.

Many investigators have previously reported that regenerative capacity of barley cultures decreases during successive subculturing (Scheunert et al., 1977; Dale and Deambrogio, 1979; Jelaska et al., 1984). These results are confirmed by this study. A decline in totiopotency was observed 6 months after culture initiation. Fewer green primordia appeared and shoots were not initiated frequently. Callus growth did continue for most cultures with a few shoots still emerging 9 months after culture establishment.

Cultures of four genotypes established 18 months earlier still demonstrated callus-forming ability. These calli are friable and often have numerous green sectors which no longer produce shoots or roots. This loss of totiopotency after several months in culture has frequently been reported for other cereal crops (Nabors 1983; Ahoowalia 1982; Heyser and Nabors 1982).

The conflicting conclusions drawn from the determination of correlation coefficients is disappointing. Predictions of regeneration potential based on callus growth and fresh weight gain does not seem to be possible. Negative correlations found between fresh weight gain and plant regeneration for the genotype survey are not encouraging. These studies do not support the conclusions of Nabors et al (1983) suggesting a positive correlation between the presence of green spots and regeneration potential.

CONCLUSIONS

The present data extend the information available on the response of barley genotypes to particular culture media, hormone supplements, and cultural growth conditions. Other studies have focused on the source of explant tissue and use of a range of concentrations of 2,4-D as a growth hormone. A distinguishing finding of this study was the successful use of Cl_3 POP as an auxin source for the production of callus capable of shoot proliferation. This growth regulator enhanced plant regeneration as well.

The source materials used for this investigation were collected with uniformity of germplasm in mind. Plants were established from breeder seed to ensure genetic purity and minimize sources of variation. A large amount of variation is present in these embryo explant cultures. Field populations of spring barley would be expected to exhibit greater genetic uniformity than what was observed in these tissue culture populations. Somaclonal variation in regenerated plant populations has been described repeatedly in cereal crop tissue culture literature (Larkin et al. 1984).

Individual genotypes have been identified for their rapid callus initiation and superior callus yielding ability. Genotypes that have demonstrated predictable shoot proliferation and regeneration potential can now be used in further applied tissue culture studies.

It was not the intent of these studies to select and subculture callus types that developed large numbers of green primordia. If callus transfers had been carried out in a selective manner the number of plants regenerated could have been increased. Specific genes for

plantlet regeneration are likely to exist. An investigation such as this which selects for genotypes with increased capacity for regeneration will help identify the factors controlling tissue culture regeneration.

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HETEROSIS IN TISSUE CULTURE POPULATIONS OF SPRING BARLEY, (*HORDEUM VULGARE*)

ABSTRACT

Evidence of heterosis in tissue culture was detected for callus growth, root and shoot initiation, and regeneration of plantlets in four spring barley crosses. Immature embryo explants were taken 8 days after anthesis to initiate tissue culture populations. For plantlet regeneration and gain in fresh weight 9 weeks after culture initiation, a heterotic effect existed for all F_1 tissue culture population means. A significant correlation between fresh weight of 9 week old calli and both shoot development and regeneration of plants was shown. The results clearly demonstrate that heterosis was present in F_1 tissue culture populations from spring barley crosses. The increase in numbers of plants regenerated suggests that F_1 materials are well suited for tissue culture studies directed towards the genetic improvement of cereal crops.

INTRODUCTION

Tissue culture populations of cereal crops are often inconsistent in their potentials for callus formation and regeneration of several plantlets from an individual explant. A system must be developed to ensure rapid and reliable plantlet regeneration before tissue culture methods can be applied to a range of crop improvement objectives. Solutions to the problem of improvement of totiopotency must be researched as a first step. The phenomenon of hybrid vigor in F_1 tissue culture populations is a promising answer for increasing the capacity for plant regeneration.

This investigation focuses on the concept of heterosis in tissue culture. The phenomena of hybrid vigor has been well documented for many traits in F_1 populations of agronomic crops. Heterosis would similarly be expected to occur *in vitro*. These F_1 populations would be likely to demonstrate measurable increases in vigor of callus production, shoot initiation, and root development. A major objective of this study was to determine whether F_1 generation cultures of barley were capable of more vigorous callus growth and more prolific plantlet regeneration, compared to their respective parents.

MATERIALS AND METHODS

Four sets of spring barley crosses were made in the greenhouse to evaluate the phenomena of heterosis in tissue culture populations. Developing spikes were bagged to prevent pollination from other pollen donors. Tissue culture populations were established from the resulting F_1 immature embryos and the respective parents used in the crosses.

Cultures were derived from immature embryo explants taken 8 days after anthesis. The caryopses were surface-sterilized for 5 minutes in a 20-fold dilution of commercial bleach (5.25% sodium hypochlorite), and rinsed five times in sterile distilled water. Embryos were excised under aseptic conditions using a dissecting microscope. Close attention was given to both the developmental age of the embryo and explant size. Each embryo taken was 0.5 to 1.0 mm in length. This ensured rapid establishment and growth of calli while avoiding precocious germination of the embryo. Embryos that showed signs of shoot and root germination in the first 4 weeks were discarded from the study.

Cultures were established on Gamborg's B5 medium (Gamborg and Shyluk, 1981) supplemented with 1.0 mg L^{-1} 2,4-D (2,4-dichlorophenoxyacetic acid). Both callus growth and shoot initiation were achieved on this medium. Roots were induced in some cultures in the presence of 2,4-D. Other cultures were transferred to auxin-free media before root development was observed.

The following set of spring barley crosses were carried out for this investigation: Akka X Klages, Clark X Morex, Lewis X Steptoe, and

OSB 763390 X Advance. Genotypes chosen as parents had exhibited superior production of callus, shoots, and roots in preliminary tests. Comparisons were made amongst the resulting F_1s using a completely randomized design.

Four traits were measured to evaluate the degree of heterosis observed in tissue culture populations. These included gain in fresh weight at 9 weeks, gain in fresh weight at 13 weeks, shoot production, root development, and plantlet regeneration. Degree of heterotic effect was determined using midparent values for comparison. Heterosis was calculated using the formula

$$\frac{F_1-(P_1+P_2)/2}{P_1+P_2/2}$$

RESULTS

No differences were detected for gain in fresh weight, root development, or regeneration of plantlets when comparisons were made among F_1s. There were significant differences in shoot development.

Varying degrees of hybrid vigor were noted for callus weight gain 9 weeks after initiation in all F_1 embryo cultures. A heterotic pattern for shoot initiation and gain in fresh weight scored 13 weeks after initiation was observed in three of the four crosses. Heterosis was also noted for regeneration of plantlets in all F_1 populations.

Progeny from the cross of OSB 763390 X Advance initiated 2.5 to 4.4 times as many shoots as any of the other F_1 cultures. Immature embryo explants originating from the cross between OSB 763390 and Advance produced cultures that were superior to the other crosses in all traits evaluated except root initiation. The cultivar Advance was not superior for these traits when compared to other parental sources. The Oregon State University breeding line 763390 produced callus cultures capable of prolific shoot development and a high frequency of plantlet regeneration.

Correlations between callus growth and shoot development were examined. Correlation coefficients were significant at the .05 level of probability for the relationship between mean gain in fresh weight of 9 week old cultures and both shoot initiation and regeneration of plantlets. The same positive correlation existed at the .10 probability level for comparisons of gain in fresh weight recorded at 13 weeks and shoot or plantlet development. Other research has shown that it is difficult to use callus growth and weight gain as a

predictor of regeneration potential for cereal tissue cultures.

Table 8. Simple correlation coefficients between four tissue culture traits in twelve spring barley populations.

	Shoot initiation (13 wks)	Regeneration (7 months)	Root initiation (13 wks)
Gain in fresh weight (9 wks)	0.58 *	0.59 *	0.21
Gain in fresh weight (13 wks)	0.57	0.53	0.36
Root initiation (13 wks)	0.11	0.26	

*: Significant at the 0.05 probability level.

1) Each correlation coefficient is based on twelve means. Four were F_1 population treatment means. Eight were from the means of their parental cultivars.

CONCLUSIONS

Heterosis was present *in vitro* in the spring barley populations examined. Greater numbers of plantlets were regenerated from F_1 immature embryo explant cultures than from their parents. The average number of regenerated plantlets for F_1 populations exceeds the number of plants recovered in studies of regenerative capacity of twenty genetically diverse spring barley genotypes.

Correlations were shown for measurements of gain in fresh weight and the regeneration of both shoots and plants. Unfortunately there is little consistency with the findings of similar studies. Gain in fresh weight can not confidently be used as a predictor of the regeneration potential of a given genotype.

Vigorous callus growth exhibited by these F_1s and the regeneration of greater numbers of plantlets suggest that F_1 populations may be a good source of materials for applied tissue culture studies in barley. Salman et al. (1984) have described heterosis for callus growth and totiopotency of cultures in winter wheat cultures. This is a promising method for increasing the numbers of plants that can be regenerated in cereal tissue culture experiments. F_1 immature embryos will be a valuable source of explant materials for applied tissue culture investigations such as in vitro selection schemes for resistance to diseases, herbicides, or mineral toxicity of soils.

Table 9. Mean heterosis values for F_1 populations of four spring barley crosses based on midparent value.

CROSS	CHARACTERISTIC MEASURED: Gain in fresh weight of callus (9 wks)		
	MP (grams)	F_1 (grams)	Heterosis
OSB 763390 X ADVANCE	.300	.804	1.677
Akka X Klages	.672	.807	.199
Lewis X Steptoe	.505	.631	.250
Clark X Morex	.662	.673	.016

CROSS	Gain in fresh weight of callus (13 wks)		
	MP (grams)	F_1 (grams)	Heterosis
OSB 763390 X Advance	.635	1.569	1.471
Lewis X Steptoe	.904	1.168	.292
Clark X Morex	1.393	1.427	.024
Akka X Klages	1.326	1.203	-.093

CROSS	Development of roots (13 wks)		
	MP	F_1	Heterosis
OSB 763390 X Advance	.5	1.4	1.743
Lewis X Steptoe	1.4	2.8	1.041
Clark X Morex	2.8	1.7	-.387
Akka X Klages	.6	.3	-.402

1) (Weeks and months) represent the number of weeks or months after cultures were initiated.

2) Formula for computation of heterosis = $\dfrac{F_1-(P_1+P_2)/2}{P_1+P_2/2}$

3) MP refers to mid-parent value.

Table 10. Heterosis calculated for F_1 populations of four spring barley crosses based on midparent value.

CROSS	CHARACTERISTIC MEASURED: Development of shoots (13 wks) MP	F_1	Heterosis
OSB 763390 X Advance	.8	11.3	13.07
Lewis X Steptoe	1.29	2.6	1.144
Clark X Morex	2.9	3.6	.219
Akka X Klages	4.7	4.3	-.077

CROSS	Regeneration of plantlets (7 mos) MP	F_1	Heterosis
OSB 763390 X Advance	.4	2.4	5.28
Lewis X Steptoe	.6	1.7	1.81
Clark X Morex	1.0	1.4	.444
Akka X Klages	1.5	2.1	.397

1) (Weeks and months) refer to the number of weeks after cultures were initiated.

2) Formula for computation of hybrid vigor = $\frac{F_1-(P_1+P_2)}{P_1+P_2/2}$

3) MP refers to mid-parent value

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APPENDIX

Table 11. Gain in fresh weight for callus cultures 4 weeks after initiation. Results gathered for hormone experiment data are presented for the cultivar Klages.

Analysis of variance

Sources	df	MS	F
Treatment	3	.036	10.202 **
Rep	5	.013	
Error	15	.004	
Total	23	.010	

*: Significant at 5% level
**: Significant at 1% level

Table 12. Gain in fresh weight for callus cultures 9 weeks after initiation. Results of hormonene treatment experiments are presented for the cultivar Klages.

Analysis of variance

Sources	df	MS	F
Treatment	3	.235	10.749 **
Rep	5	.076	
Error	15	.022	
Total	23	.061	

*: Significant at 5% level
**: Significant at 1% level

Table 13. Measurements of green primordia appearance 9 weeks after initiation. Results gathered for hormone treatment experiments are presented for the cultivar Klages.

Analysis of variance

Sources	df	MS	F
Treatment	3	.758	.932 (NS)
Rep	5	.519	
Residual	15	.813	
Total	23	.742	

*: Significant at 5% level
**: Significant at 1% level
NS: Not statistically significant

Table 14. Occurrence of shoots for cultures 9 weeks after initiation. Results of hormone treatment experiments are presented for the cultivar Klages.

Analysis of variance

Sources	df	MS	F
Treatment	3	.235	.376 (NS)
Rep	5	.700	
Error	15	.623	
Total	23	.589	

*: Significant at 5% level
**: Significant at 1% level

Table 15. Occurrence of primordia for cultures 12 weeks after initiation. Results of hormone treatment experiments are presented for the cultivar Klages.

Analysis of variance

Sources	df	MS	F
Treatment	3	1.535	.900 (NS)
Rep	5	1.805	
Error	15	1.706	
Total	23		

*: Significant at the 5% level
**: Significant at the 1% level
NS: Not statistically significant

Table 16. Development of shoots for cultures 12 weeks after initiation. Results gathered for hormone treatment experiments are presented for the cultivar Klages.

Analysis of variance

Sources	df	MS	F
Treatment	3	2.352	1.087 (NS)
Rep	5	2.941	
Error	15	2.163	
Total	23		

*: Significant at 5% level
**: Significant at 1% level
NS: Not statistically significant

Table 17. Development of roots for cultures 12 weeks after initiation. Results of hormone treatment experiment data are presented for the cultivar Klages.

Analysis of variance

Sources	df	MS	F
Treatment	3	62.98	19.99 **
Rep	5	2.54	
Error	15	3.15	
Total	23		

*: Significant at 5% level
**: Significant at 1% level

Table 18. Regeneration of plantlets for cultures 6 months after initiation. Results of hormone treatment experiment data are presented for the cultivar Klages.

Analysis of variance

Sources	df	MS	F
Treatment	3	2.449	20.50 **
Rep	5	.127	
Error	15	.119	
Total	23		

*: Significant at 5% level
**: Significant at 1% level

Table 19. Hormone treatment means for root development of callus cultures 12 weeks after initiation for the cultivar Klages.

TREATMENT (mg L^{-1})	NUMBER OF ROOTS
Cl_3 POP 1.0	8.1 a
CL_3 POP 2.5	4.6 b
2,4-D 1.0	1.8 c
Cl_3 POP 5.0	0.9 c

1) Mean of six replications.
2) Means with a letter in common are not significantly different. $LSD_{.05}$ for comparison among these means = 2.18.

Table 20. Gain in fresh weight for cultures 6 weeks after initiation. Results of genotype evaluations are presented for 20 spring barley cultivars.

Analysis of variance

Sources	df	MS	F
Treatment	19	.113	7.152 **
Rep	4	.023	
Error	76	.016	
Total	99		

*: Significant at 5% level
**: Significant at 1% level

Table 21. Development of shoots for cultures 12 weeks after initiation. Results of genotype evaluations are presented for 20 spring barley cultivars.

Analysis of variance

Sources	df	MS	F
Treatment	19	15.62	2.57 **
Rep	4	9.73	
Error	76	6.08	
Total	99		

*: Significant at 5% level
**: Significant at 1% level

Table 22. Development of roots for cultures 12 weeks after initiation. Results of genotype evaluations are presented for 20 spring barley cultivars.

Analysis of variance

Sources	df	MS	F
Treatment	19	7.94	1.96*
Rep	4	14.11	
Error	76	4.04	
Total	99		

*: Significant at 5% level
**: Significant at 1% level

Table 23. Regeneration of plantlets for cultures 6 months after initiation. Results of genotype experiment evaluations are presented for 20 spring barley cultivars.

Analysis of variance

Sources	df	MS	F
Treatment	19	1.803	2.84 **
Rep	4	.416	
Error	76	.635	
Total	99		

*: Significant at 5% level
**: Significant at 1% level

Table 24. Gain in fresh weight for callus cultures 9 weeks after initiation. Results of heterosis studies are reported for four F_1 spring barley crosses.

Analysis of variance

Sources	df	MS	F
Treatment	3	.049	.399 (NS)
Error	20	.122	
Total	23	.112	

*: Significant at 5% level
**: Significant at 1% level
NS: Not statistically significant

Table 25. Gain in fresh weight for callus cultures 13 weeks after initiation. Results of hererosis studies are reported for four F_1 spring barley crosses.

Analysis of variance

Sources	df	MS	F
Treatment	3	.217	.869 (NS)
Error	20	.250	
Total	23	.246	

*: Significant at 5% level
**: Significant at 1% level
NS: Not statistically significant

Table 26. Development of shoots for callus cultures 13 weeks after initiation. Results of heterosis study are presented for four F_1 spring barley cultivars.

Analysis of variance

Sources	df	MS	F
Treatment	3	95.65	5.95 **
Error	20	16.11	
Total	23	26.49	

*: Significant at 5% level
**: Significant at 1% level
NS: Not statistically significant

Table 27. Treatment means for shoot development 13 weeks after culture initiation for four spring barley crosses.

CROSS	NUMBER OF SHOOTS
OSB 763390 X Advance	11.3 a
Akka X Klages	4.3 b
Clark X Morex	3.6 b
Lewis X Steptoe	2.6 b

1) Mean of shoot development for six replications.
2) Means with a letter in common are not significantly different. $LSD_{.05}$ for comparison among these means = 4.83.

Table 28. Development of roots for callus cultures 13 weeks after initiation. Results of heterosis studies are presented for four F_1 spring barley crosses.

Analysis of variance

Sources	df	MS	F
Treatment	3	6.05	1.18 (NS)
Error	20	5.14	
Total	23	5.26	

*: Significant at 5% level
**: Significant at 1% level
NS: Not statistically significant

Table 29. Regeneration of plantlets 7 months after culture initiation. Results of heterosis studies are presented for four F_1 spring barley crosses.

Analysis of variance

Sources	df	MS	F
Treatment	3	1.12	.693 (NS)
Error	20	1.61	
Total	23	1.55	

*: Significant at 5% level
**: Significant at 1% level
NS: Not statistically significant