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EFFECT OF GENOTYPES AND NUTRIENT MEDIA ON IMMATURE EMBRYO CULTURE OF WHEAT

D. K. AGARWAL AND SHARAD TIWARI

Plant Tissue Culture Laboratory, Department of Plant Breeding & Genetics
Jawaharlal Nehru Krishi Vishwa Vidyalaya
Jabalpur 482004

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ABSTRACT

Immature embryo of 14 genotypes of *Triticum aestivum* and 4 genotypes of *T. durum* were cultured on four modified MS medium supplemented with 30 $g.l^{-1}$ sucrose, 8 $g.l^{-1}$ agar and different level of growth hormones, viz. 2 $mg.l^{-1}$ 2,4-D; 5 $mg.l^{-1}$ 2,4-D; 2 $mg.l^{-1}$ 2, 4- D + 100 $ml.l^{-1}$ coconut milk; and 2 $mg.l^{-1}$ 2,4-D + 0.5 $mg.l^{-1}$ kinetin. Highly significant differences in the response of genotypes, culture media and genotype x medium interactions were observed for callus initiation from immature embryos, formation of embryogenic calli and plantlet regeneration.

Key words: Embryo culture, genotype x medium interaction, wheat, *T. aestivum*, *T. durum*.

In wheat, in vitro culture technology has attained considerable attention in recent years for genetic improvement. To attain specific objectives employing in vitro technology, it is always important to have a highly efficient and productive culture system. In monocots, explants with meristematic cells develop callus and are competent to express totipotency. In wheat, the immature embryos produce regenerable callus most efficiently. Embryo culture is now being used extensively and has become the most suitable technique to obtain incompatible interspecific and intergeneric hybrids by embryo rescue [1, 2], to raise somaclonal variations [3–7], to recover haploid plants after interspecific and intergeneric crosses [8, 9], for in vitro selection [10, 11] and for isolation of totipotent protoplasts from embryogenic culture [12, 13].

In wheat, the factors that influence embryo culture the most are genotype and nutrient medium [14–18]. In this report, we describe genotypic influences and determine effects of medium composition on totipotent callus induction and plant regeneration from immature embryos of wheat.

MATERIALS AND METHODS

Fourteen genotypes of *Triticum aestivum* and 4 genotypes of *Triticum durum* used in the study were either established cultivars or advanced breeding lines developed at Wheat Research Station, Department of Plant Breeding and Genetics, J.N.K.V.V., Jabalpur.

Dehusked immature seeds (14–18 days post-anthesis) were treated with 70% ethanol for 1 min and with 1% $AgNO_3$ for 5 min, followed by 5 washings with sterile distilled water. Immature embryos (1–2 mm long) were excised aseptically and cultured on four different media. The basal MS medium [19] was supplemented with 2.0 $mg \cdot l^{-1}$ 2,4-D (MS-2D); 5.0 $mg \cdot l^{-1}$ 2,4-D (MS-5D); 2.0 $mg \cdot l^{-1}$ 2,4-D + 10% coconut milk (MS-DC); and 2.0 $mg \cdot l^{-1}$ 2,4-D + 0.5 $mg \cdot l^{-1}$ kinetin (MS-DK) to make four different media. All the media were supplied with 30 $g \cdot l^{-1}$ sucrose and adjusted to pH 5.8 prior to the addition of 8.0 $g \cdot l^{-1}$ agar. Each 90 x 15 mm Petri dish was plated with 25 embryos keeping the scutellum in upward position. Immature embryos collected from one spike were evenly distributed on the four culture media. The cultures were stored at 20–25°C with 16 h light/8 h dark cycle under dim light.

After 35 days, observations were recorded for calli initiation and formation of embryogenic calli out of the embryos plated. The calli were then subcultured on MS medium supplemented with 1 $mg \cdot l^{-1}$ IAA, 1 $mg \cdot l^{-1}$ BA, 15 $g \cdot l^{-1}$ sucrose and 8 $g \cdot l^{-1}$ agar for regeneration. The data were analysed in factorial completely randomized design.

RESULTS

CALLUS INITIATION

The first response of the immature embryos to culturing was similar after 4–6 days and mostly independent of genotypes or culture media. All the embryos became swollen and enlarged, and no callus proliferation was observed. During the second week of culture, callus formation was observed from scutellar part of the embryo. Shoot formation from the embryonal axis of few immature embryos with little callus formation was also observed. Only callus initiating embryos were counted after 5 weeks of culture (Table 1).

Although callus initiation was recorded in all the genotypes in the four culture media tested, its frequency varied among genotypes as well as in culture media. Significant genotype x culture medium interactions were also recorded. More than 50% of the cultured immature embryos of 10 out of 18 genotypes initiated callus on various media. Maximum of callus initiation (69.5%) was recorded in JWJ 90-B-25. The other genotypes with high callus induction (> 60%) were Sonalika, JWJ 68, JWJ 90-B-11, A 9-30-1, and HD 4530.

Table 1. Number of callus forming embryos, number of embryogenic calli and number of plants obtained per 100 immature embryos plated on different media

Genotype	Callus forming embryos (%)					No. of embryogenic calli per 100 embryos					No. of plants obtained per 100 embryos				
	MS-2D	MS-5D	MS-DC	MS-DK	mean	MS-2D	MS-5D	MS-DC	MS-DK	mean	MS-2D	MS-5D	MS-DC	MS-DK	mean
Lok-1	51.5	45.0	48.0	46.5	45.2	38.0	32.5	34.5	35.0	35.0	13.0	10.5	18.0	13.5	13.7
Hyb. 65	62.5	52.0	52.5	41.5	52.1	44.0	36.5	38.5	27.5	36.6	17.0	18.5	23.5	20.5	19.9
HI 1077	55.5	48.5	63.5	56.5	56.0	37.0	25.5	46.5	38.5	36.9	22.5	17.5	24.5	13.5	19.5
Sonalika	69.0	59.0	61.0	73.5	65.6	46.0	38.0	41.0	49.0	43.5	16.5	21.5	26.5	21.0	21.4
JWJ-16	34.5	47.0	37.5	52.0	42.7	33.5	22.0	24.5	26.5	26.6	17.0	11.0	13.5	10.5	13.0
JWJ-68	67.5	66.0	57.5	60.5	62.9	47.0	45.0	39.0	40.5	42.9	20.0	17.0	15.0	20.0	18.0
JWJ-90-B-11	69.5	61.0	53.5	57.5	60.4	49.5	44.5	34.5	38.5	41.7	20.5	11.0	18.5	18.0	14.5
JWJ-90-B-14	10.0	31.0	19.0	22.5	20.6	7.0	23.5	13.5	16.5	15.1	9.5	1.5	6.0	5.5	5.6
JWJ-90-B-25	65.5	76.5	69.5	66.5	69.5	44.5	53.0	47.5	42.5	46.9	36.0	37.0	44.5	42.5	40.0
JWJ-90-B-39	55.5	61.5	49.5	51.0	54.4	41.0	43.0	33.5	36.5	38.5	18.5	18.0	10.5	13.0	15.0
JWJ-90-B-44	52.5	56.0	60.5	46.5	53.9	34.0	37.5	42.5	28.0	35.5	15.5	12.0	16.0	19.5	15.7
JWJ-90-B-38-4	56.5	52.0	53.5	51.0	53.2	39.0	33.0	34.0	32.0	34.5	18.0	12.0	14.5	13.0	14.4
JWJ-90-B-38-8	40.0	44.5	42.5	31.5	39.6	21.5	26.5	23.0	21.0	23.0	5.0	7.0	3.5	8.0	5.9
JWJ-90-B-38-13	54.5	61.5	50.0	52.5	54.6	36.0	46.5	35.5	52.0	42.5	21.5	15.5	14.5	21.5	18.2
Jayraj	23.0	30.0	29.5	20.5	25.7	15.0	20.5	20.5	14.5	17.6	10.5	7.5	8.0	7.5	8.4
A-9-30-1	57.0	65.5	65.5	59.5	61.9	36.5	42.0	43.5	37.5	39.9	32.5	23.0	27.5	41.0	31.0
HD 4530	63.5	73.5	61.0	58.5	64.1	43.5	49.5	41.5	39.5	43.5	35.5	26.5	29.5	34.0	31.4
Meghdoot	54.5	49.5	46.0	45.0	48.7	37.5	37.0	32.0	28.5	31.2	5.0	10.0	7.5	12.0	8.6
Mean	52.35	54.5	51.11	49.05		35.53	36.44	34.75	33.55		18.55	15.93	17.83	18.58	

Among the culture media, MS-5D gave highest callus initiation (54.5%), followed by MS-2D, MS-DC and MS-DK. On medium MS-5D, maximum immature embryos of genotypes JWJ 90-B-25 and HD 4530 developed callus (76.5 and 73.5%, respectively); while on MS-2D, nearly 70% embryos of these two genotypes showed callus initiation. Maximum callus initiation (73.5%) in cv. Sonalika was observed on the medium MS-DK which gave the overall lowest performance.

EMBRYOGENIC CALLUS FORMATION

During the third week of culture, the calli could be distinguished on the basis of their morphological features. Most of the calli were compact and yellowish white. The other types of calli obtained were lose, soft, friable and translucent. After 5 weeks of culture, well organized highly lobed regions were apparent in many of the compact yellowish white calli. Such calli were taken as embryogenic calli.

Significant variations were observed among the genotypes, culture media and genotype x medium interactions for their ability to produce embryogenic calli. More than 40% immature embryos 6 genotypes formed embryogenic calli, the maximum being in the genotype JWJ 90-B-25. Among the culture media, MS-5D supported maximum somatic embryogenesis, followed by MS-2D, MS-DC and MS-DK.

PLANT REGENERATION

All the calli transferred from the four induction media to the regeneration medium formed plantlets within 14–21 days. Plant regeneration occurred either via somatic embryogenesis or through gemmo-rhizogenesis. Plantlets with 2–3 leaves and with or without small primary roots were transferred to culture vessels with half strength MS medium for root development. After 3–4 weeks of culture, the plantlets with well developed root system were transferred to the pots.

A wide range of difference was observed among genotypes, culture media and genotype x medium interactions for plant regeneration potential. Genotype JWJ 90-B-25 showed highest mean regeneration frequency of 40% over all the media. More than 30% regeneration from the cultured embryos was recorded in the two durum cultivars, A 9-30-1 and HD 4530.

Among the four media used for callus initiation, MS-DK and MS-2D gave maximum plant production. Although maximum embryogenic callus formation was observed in the medium MS-5D, more plant regeneration was observed from the calli which developed on MS-2D and MS-DK. The medium-MS-2D produced more somatic embryos per callus which,

in turn, regenerated more plantlets. Medium MS-DK showed the lowest callus initiation and embryogenic calli formation, but facilitated regeneration through gammo-rhizogenesis.

DISCUSSION

The field grown wheat plants were used to obtain 14–18 days old embryos for culture. At this stage of embryo development, high frequency of plant regeneration has been accomplished by various workers [18, 20–22]. The immature embryos were cultured with the scutellum facing upward as it has been reported to provide better response [14–16, 18].

A wide variability among genotypes was recorded for response to culture. It has been reported that regeneration under culture is generally controlled by nuclear genes in maize [23, 24]. Other studies have also suggested that at least one gene or a block of genes controls somatic embryogenesis in maize tissue cultures [25]. In sorghum, the ability to form regenerable callus varied among genotypes as a dominant trait with at least two gene pairs [26].

Genotypic differences may be related to variations in the endogenous hormone levels [27]. Immature embryos collected from the same inflorescence behave differently in culture, depending on their size and location in the inflorescence. Response of explants from a well nourished plant is different from those of a nutrient-starved plants [28]. The cultures from the plants grown in summer give different response from those collected from plants grown in the cool season [26]. In the present investigation, immature embryos were collected for culture from plants growing in field. It is reasonable to assume that the differences in response to culture result from the genetical differences among genotypes.

As is known, composition of basal medium does not play a major role in deciding the in vitro response as the type and concentration of growth hormones [29]. Sensitivity to growth hormones is probably determined by the endogenous levels of hormones in the cells [30]. Thus the genetic basis of variability in tissue culture response and morphogenesis is most likely due to differences in hormone metabolism within the explant which is established by the level of gene expression for individual hormones by the genotype. Significant differences in genotype x medium interaction observed in the present investigation may be improved by modifying the culture medium further.

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