Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2017; 6(5): 2658-2661
Received: 16-07-2017
Accepted: 17-08-2017

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A new method to increase callus induction and plant regeneration from mature embryo of wheat

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Abstract
Callus cultures were initiated on MS medium supplemented with 7mg/*l* 2,4-D+0.2 mg/1 NAA from mature embryos of nontreated, non-endosperm supported (NS) and nontreated, 30 kR and 35 kR gamma irradiated endosperm supported mature embryos (ES, 30 kR, ES and 35 kR, ES). Callus initiation took place within 2 days after inoculation from ES system (ES, 30 kR and 35 kR, ES) while it took 6 days in NS system. Callusing was highest in NS mature embryos (79.9%) among the all [ES (72.2%), 30 kR, ES (76.3%) and 35 kR, ES (74.7%)]. After 10 days the developed calli were transferred to MS+5mg// 2,4-D medium and subcultured at every two week interval for 5 months. Regeneration took place within 6 days in all types of calli on MS+0.1 mg/*l* NAA medium. Regeneration was best in 30 kR, ES embryo derived calli (88.4%) among the all [NS (83.2%), ES (70.5%) and 30 kR, ES (76.9%) Healthy and well developed regenerated plants were transferred to soil in pots. Survival was 80 percent. Gamma irradiation exhibited positive effect for callusing as well as regcnaration.

Keywords: Gamma irradiation, wheat, mature embryo, callusing and regeneration

Introduction
The establishment *of in-vitro* regenerable system plays a significant role in the biotechnological improvement of cereals. In wheat, *in-vitro* cultures have to be initiated from immature tissues like immature embryos [1] and immature inflorescences [2]. Mature differentiated tissues cannot generally be induced to reenter cell division and proliferate. The growth of mature donor plants for a regular supply of immature embryos and inflorescences is not only labour intensive but also time and space consuming. Also these explants are available in the field only within limited period of the year. Mature embryos, which can easily store and are readily available at all times are the less frequently used explant sources due to their late callus initiation and low frequency of callus induction and regeneration. However some new techniques such as endosperm-supported mature embryo cultures have been successfully used [3, 4]. The purpose of present study was to determine effect of mutagen, mutagenic different doses and endosperm metabolities on mature embryo cultures and to compare with nontreated nonendosperm metabolites supported mature embryo in wheat.

Materials and methods
Treated seeds with 30 kR and 35 kR doses of gamma irradiation and nontreated seeds of different six wheat cultivars were used as a source of mature embryos. Tween 80 washed seeds were soaked overnight. Mature embryos were excised from the overnight presoaked seeds after sterilization with 1% $HgCl_2$ for 8 min and washed with sterile distilled water (5 times). Mature embryos of nontreated and gamma rays treated seeds were cultured on MS [5] medium containing 7mg/*l* 2, 4-D+0.2mg/*l* NAA. Mature embryos from gamma rays treated (30 kR and 35 kR) and nontreated seed were aseptically mov-ed (not set free) slightly (ES) or remain attached with the seed. The seeds with slightly moved embryos in the imbibed seeds (furrow downwards) were placed on medium. While completely removed mature embryo (NS) of nontreated seeds were also culture on the same medium. After 10 days, the developed calli of all types were subcultured on MS+5mg/*l* 2, 4-D medium for 5 month at every two weeks interval and then transferred to MS+0.1mg/*l* NAA medium for regeneration. Well-developed regenerated plants were transferred to soil in pots and grown to maturity to collect the seeds.

Culture conditions
All the media were adjusted to pH $5.8^{\pm 1}$ before autoclaving. The cultures were kept at $25^{\pm 10}$C under dark conditions for callusing and under a continuous light about 1400 lux intensity of 16 h photo period (white fluorescent light) for regeneration.

Statistical analysis

Test tubes containing about 36 mature embryos attached with seeds (Endosperm supported) and mature embryos without seeds (non-endosperm supported) for each genotype (3 embryos per test tube) were considered the unit of replication, as each experiment had three replication. Data were analysed by using Completely Randomized Design (CRD) after angular transformation of data.

Results and Discussion

Callus formation took place within 2 days after inoculation from endosperm supported mature embryos (ES) of nontreated and gamma irradiated (30 kR and 35 kR seeds) in all genotypes. Callus was whitish and watery (Fig. 1) callus induction was highest in 30 kR, ES (76.3%) mature embryo among ES (72.2%) and 35 kR, ES (74.7%) on overall mean basis (Table 2). UP2338 exhibited highest callusing (87.9%) among all cultivars in ES system. After 10 days of inoculation, size of the callus reached or surpassed that of the seeds and they became suitable for sub-culture (Fig. I). Nevertheless, callus size varied according to the genotype and Raj 3765 was best in this respect followed by DI 9 in all cases (ES, 30 kR, ES and 35 kR, ES). The percentage range of nonendosperm supported mature embryos (NS) for callus induction was 56.6 to 100 percent and highest was obtained from UP 2338 (100%). Callus was compact white, nodular and embryogenic. Callusing was significantly highest in NS (79.9%) among ES (72.2%), 30 kR, ES (76.3%) and 35kR, ES (74.7%). While callus growth was better in all ES system with early callus initiation (after 2 days) in comparison to NS system (after 6 days). Significant genotypic differences were also observed for callus induction among different systems (NS, ES, 30kR ES, 35kR ES), Regeneration look place with in 6 days when calli of all types were transferred to regeneration medium (Fig 2). Regeneration was significantly highest in 30kR ES calli (88.4%) among the all NS (83.2%), ES (70.5%) and 35 kR ES (76.9%) on over all mean basis and 30 kR, ES calli of PBW 343 showed highest regeneration (92.5%) (Table 3). Healthy and well developed regenerated plants were transferred to soil in pots (Fig.3) and grown to maturity to collect the seed for further studies in field to evaluate somaclonal variation. Significant differences were also observed for genotype x treatments for callusing as well as regeneration. Results revealed callus induction up to 100 percent and regeneration up to 92.5 percent. However, Sidana *et al*. [6] reported callusing range of 20.3 to 93.0 percent and regeneration from 2.8 to 30 1'crcenl only. However, callusing percentage significantly higher in NS system but mature embryo derived calli produced by the ES system seem to be suitable as it provides earliest callus initiation, rapid callus growth and highest regeneration (30 kR, ES). Bartok and Sagi [3] and Ozgen *et al*. [4] also used the endosperm supported system and found good results but no comparative studies between NS and ES mature embryo cultures were made. In present study 30 kR gamma irradiation showed significantly positive effect for callusing among P'S mature embryos and for regeneration among the all (NS, ES and 35 kR, ES). Gao [7] also reported that 30 kR gamma rays treated seeds derived immature embryo showed higher callusing and regeneration than untreated one. While Abdrabou [8] observed that irradiation of seeds before sowing in field, decreased callus formation (cv. Giza 155) using immature embryos of these wheat plants and plantlct formation (cv. Sakha 8) also. While (Hu 9) reported that if calli were irradiated with gamma rays, cell ultra-structure was seriously damaged at 5000 R gamma rays and eventually resulted in death of cells. Thus in all the aspects, endosperm supported mature embryos if treated with gamma rays (30 kR dose) proved to be the best. The usefulness of this system (ES with gamma irradiation) can perhaps be extended to other cereals and millets also.

Table 1: Analysis of variance and the meansquares for callus induction and regeneration in wheat mature embryo.

S.O.V.	Callusing		Regeneration	
	D.f.	M.S.	D.f.	M.S.
Factor A	03	194.79	03	708.56
Factor B	05	307.37	05	77.04
Factor AxB	15	254.72	15	99.49
Error	48	18.27	48	45.97

A - Treatment
B - Genotype

Table 2: Comparison of callus induction frequency between NS and ES mature embryos and among ES non-treated, ES with 30 kR and ES with 35 kR treated mature embryo of different wheat genotypes

Genotype	Non-treated mature embryo (NS)	Non-treated Mature embryo (ES)	30 kR treated mature embryo (ES)	35 kR treated mature embryo (ES)
DI 9	72.00 (58.74)	66.4 (54.70)	74.3 (59.60)	76.8 (61.10)
UP 2338	100.00 (90.00)	87.69 (69.83)	84.9 (66.89)	70.3 (57.7)
Raj 3765	70.3 (57.10)	86.6 (68.85)	69.2 (56.32)	88.6 (70.97)
WH 147	88.8 (70.49)	62.9 (52.52)	69.0 (56.34)	79.5 (62.92)
PBW 343	91.7 (73.29)	70.3 (57.07)	73.4 (59.08)	62.9 (52.50)
WH 542	56.6 (48.93)	59.2 (50.35)	87.3 (69.64)	70.3 (57.02)
Total Mean	79.90 (66.43)	72.22 (58.89)	76.35 (61.31)	74.73 (60.27)
CD	A	2.87		
	B	3.51		
	AxB	7.02		

Figures in parentheses are angular transformed values

Table 3: Shoot regeneration responses of mature embryo calli derived from different systems (NS, ES, 30 KR ES and 35 KR ES) in different wheat genotypes.

Genotype	Non-treated mature embryo (NS)	Non-treated Mature embryo (ES)	30 kR treated mature embryo (ES)	35 kR treated mature embryo (ES)
DI 9	78.03 (62.58)	83.3 (65.99)	83.3 (66.15)	75.0 (60.51)
UP 2338	78.03 (62.06)	60.2 (50.92)	90.0 (75.0)	86.6 (68.85)
Raj 3765	86.50 (68.54)	76.16 (60.86)	90.5 (76.20)	72.3 (58.54)
WH 147	85.13 (67.46)	52.3 (46.49)	87.5 (69.68)	75.0 (60.02)
PBW 343	91.4 (76.11)	79.6 (63.78)	92.5 (76.95)	70.6 (57.19)
WH 542	80.5 (63.92)	71.5 (57.78)	86.6 (68.81)	82.2 (65.36)
Total Mean	83.27 (66.78)	70.51 (54.64)	88.4 (72.13)	76.95 (61.74)
CD	A	4.55		
	B	NS		
	AxB	11.13		

NS = Non endosperm supported system; ES = Endosperm supported system.
Figures in parentheses are angular transformed values

Fig 1: Callus induction from endosperm supported mature embryo (slightly moved mature embryos with seeds).

Fig 2: Shoot regeneration from mature embryo calli.

Fig 3: Regenerated plant transferred to soil in pots.

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