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Synergistic effect of growth regulators and glutamine on regeneration response in high yielding cultivars of wheat (*Triticum aestivum* L.)

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Abstract

The role of growth regulators and glutamine amino acid on regenerative ability from young and old callus cultures of high yielding cultivars of wheat was studied. Callus was induced from immature embryo explants of 1-1.5 mm diameter on Murashige and Skoog (MS) basal medium supplemented with 2 mg/l 2, 4-D. Shoot formation was obtained from young calli (10-12 week old) on MS medium without growth regulators. From young calli increase in regeneration frequency was obtained when cytokinin concentration was increased from 1 to 2 mg/l. The addition of auxin to cytokinin containing media further increased the regeneration frequency. When the medium contained 2mg/l cytokinin and 0.5 mg/l NAA, an average regeneration frequency of 94% was obtained. Regeneration from old calli (28-30 week old) was obtained only when the medium contained growth regulators. There was a progressive increase in regeneration frequency with the increase in cytokinin concentration from 1 to 3 mg/l. The same cytokinin medium when suplemented with low levels of auxin showed 3-6% increase in regeneration frequency. There was pronounced effect of glutamine on old callus cultures in increasing the regenerative ability by 6 to 10% when cytokinin or cytokinin- auxin containing media were supplemented with glutamine. Shoot induction frequency of 55% could be obtained from old callus cultures in a medium containing 3 mg/l cytokinin, 0.5 mg/l auxin and 150 mg/l glutamine.

Key words : Wheat, callus, glutamine, growth regulators, regeneration

Introduction

Regeneration of plants from callus is a necessary prerequisite for biotechnological approaches in crop improvement. Successful regeneration has been achieved in wheat from various explants but immature embryos are far more responsive than any other explant in culture and have been successfully utilized [1-4]. The success of regeneration in any crop depends upon the type of medium used in each phase of culture from callus initiation to maintenance and during regeneration. Media modified with growth regulators, especially 2, 4 dichlorophenoxy acetic acid (2, 4-D), have profound effect on callus induction ability and regeneration frequency [5, 6]. The shoot forming ability may be improved by the addition of kinetin in medium containing 2, 4-D or dicamba [7], a combination of auxin and zeatin [2] or auxin and benzyl amino purine [4]. The frequency of embryogenic callus formation has been shown to increase when the basal medium was supplemented with complex substances like casein hydrolysate [8] and glutamine [6, 9]. The present investigation reports the individual and synergistic effect of different growth regulators and the role of glutamine in regeneration ability from both young and old callus cultures of high yielding Indian cultivars of wheat.

Materials and methods

Experimental material comprised of two wheat cvs. CPAN3004 and PBW 226. In this study two best responding genotypes were taken on the basis of earlier report by Rao and Chawla [6] where eight genotypes were studied for regeneration. Immature embryo explants of 1.0 to 1.5 mm diameter were collected from spikes of 10-18 days after anthesis. Caryopses were surface sterilized with commercial sodium hypochlorite solution (1% active chlorine) for 10 min followed by washing with sterile distilled water 3-4 times. Immature embryos were cultured on basal medium of MS [10] supplemented with 2 mg/l 2, 4-D for callus induction. Embryos were inoculated with scutellar axis facing up and plumule - radicle axis in contact with the medium. Compact and nodular calli were considered as embryogenic and subcultured on the same medium at 4 week interval. Calli of 10-12 weeks (young) and 28-30 weeks (old) were used for the regeneration studies. Pieces of compact calli were put in different regeneration media containing cytokinins as benzyl amino purine (BAP)

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and zeatin (Zea) and naphthalene acetic acid (NAA) as auxin and glutamine (Glu) amino acid. The cultures were kept in the dark at 25°C during callus induction and maintenance, while during regeneration the cultures were incubated at 25°C with a 16/8-h photoperiod and a light intensity of 3000 lux. Data on percent shoot induction was recorded.

Results and discussion

Role of growth regulators

Regeneration from young calli - Callus could be induced from both the genotypes on MS medium supplemented with 2 mg/l 2, 4-D. The young calli of 10-12 weeks were studied for regeneration on media containing different cytokinins alone or in combination with NAA auxin. Regeneration was observed even without the addition of growth regulators in the medium but with a low frequency of 27 and 42% in PBW226 and CPAN3004 genotypes respectively (Table 1). Media composition with different growth regulators showed high frequency of shoot induction with a range of 52-100%. Significant differences were observed for regeneration ability with respect to media without growth regulators and with different concentrations of growth regulators. However, media with concentration of different cytokinin growth regulators (BAP or zeatin) did not show significant differences amongst themselves. Media supplemented with 1 mg/l of either BAP or zeatin showed regeneration frequency in the rage of 52-58% with an average of 56%. With the increase in cytokinin concentration to 2 mg/l, the regeneration frequency over the genotypes for BAP and zeatin containing media was 70%. Thus there was 14% increase in regeneration frequency with the increase in cytokinin concentration. For young calli when media containing 1 mg/l BAP or zeatin was supplemented with 0.2 mg/l of auxim, there was on an average increase of 28% in regeneration frequency of young calli. The percent regeneration for genotypes varied from 82-87%. However, when the medium contained 2 mg/l of cytokinin and 0.5 mg/l of auxin, it showed regeneration frequency of 98 and 90% for BAP and zeatin containing media respectively. CPAN3004 and PBW226 showed 100 and 97% regeneration frequency in media supplemented with 2 mg/l BAP and 0.5 mg/l NAA. Thus, there was an increase of 14 to 16% regeneration frequency in media with cytokinin and low levels of auxin when compared with media containing only cytokinin. The interaction between genotype and different media also showed some significant differences. The number of shoots per callus varied from 2 to 15.

Table 1. Regeneration reponse of immature embryo derived young calli on media with different growth regulator concentration in two cultivars of wheat

Media	CPAN3004		PBW226		Mean
	No. of calli	Regene rating calli	No. of calli	Regene rating calli	Regene rating calli
BM	12	41.6	11	27.3	34.8
BM + BAP(1)	25	56.0	21	57.1	56.5
BM + Zea(1)	23	52.2	24	58.3	55.3
BM + BAP (2)	29	72.4	25	68.3	70.4
BM + Zea (2)	26	69.2	28	71.4	70.4
BM + BAP (1) + NAA (0.2)	23	86.9	23	82.6	84.8
BM + Zea(1) + NAA (0.2)	33	84.8	29	82.7	83.9
BM + BAP (2) + NAA (0.5)	36	100.0	33	96.9	98.5
BM + Zea(2) + NAA (0.5)	27	92.6	34	88.2	90.2

$CD_G = 4.38$; $CD_M = 9.28$; $CD_{GxM} = 3.09$

In the media column : BM represents basal medium of Murashige and Skoog and values in parentheses represent concentration of growth regulators in mg/l. Genotype and mean column: values in parentheses represent percent regeneration.

Regeneration from old calli - Cell or callus lines when subcultured over a long period lose regeneration ability. However, during *in vitro* selection and genetic transformation studies, regenerative ability from old callus lines is a prerequisite. There was no regeneration when media did not contain growth regulators. Statistically the media and genotype response showed significant differences. When 1 mg/l BAP was added to the basal medium, the percentage of regeneration was 11.5% and 5% in CPAN3004 and PBW226 genotypes respectively with an average of 8.7% (Fig. 2). There was progressive increase in frequency of regeneration to 39.6% with the increase in BAP concentration to 3 mg/l. The medium containing 1 mg/l BAP and glutamine showed 50% regeneration frequency over the genotypes. The medium containing 1 or 2 mg/l BAP when supplemented with low levels of auxin

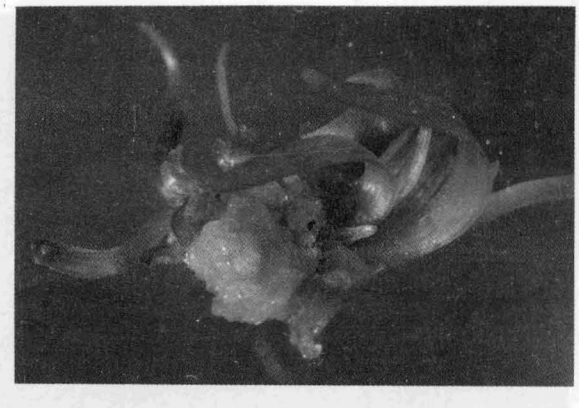

Fig. 1. Shoot regeneration from 12 week old young callus of wheat

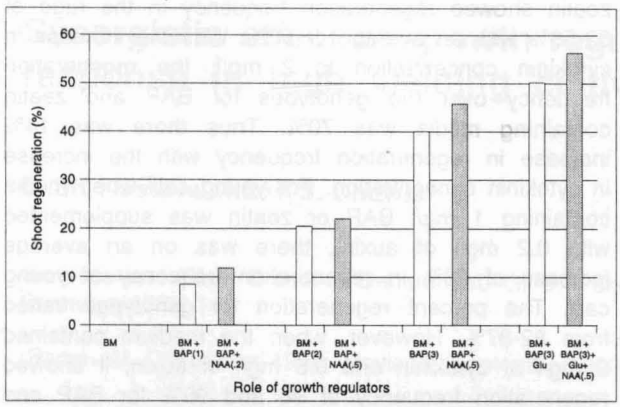

Fig. 2. The role of growth regulators for shoot regeneration in immature embryo derived old callus cultures of wheat. (BM indicates basal medium of Murashige and Skoog and values in parentheses indicate concentration of growth regulators in mg/l)

showed 1-3% increase in regeneration frequency over the medium containing cytokinin only. However, the regeneration frequency increases by 6% when the medium with 3 mg/l BAP or 3 mg/l BAP and glutamine were supplemented with 0.5 mg/l NAA. Thus, maximum frequency of regeneration was observed on medium, which contained 3 mg/l BAP, 0.5 mg/l NAA and 150 mg/l glutamine. Number of shoots per callus piece ranged from 1 to 7 (Fig. 3). It was observed that high regeneration frequency was obtained from young calli of different genotypes even without growth regulators in the medium. But growth regulators and other complex substances are required for regeneration from old calli. By increasing the concentration of cutokinin in the media from 1 to 2 mg/l for young calli and from 1 to 3 mg/l for old calli, regeneration percentage can be increased. Addition of auxin at low levels to cytokinin containing media further increased the regeneration potentiality. The influence of cytokinin alone or in combination with low levels of auxin in increasing the regeneration ability has been demonstrated in various species [4, 6, 11-13].

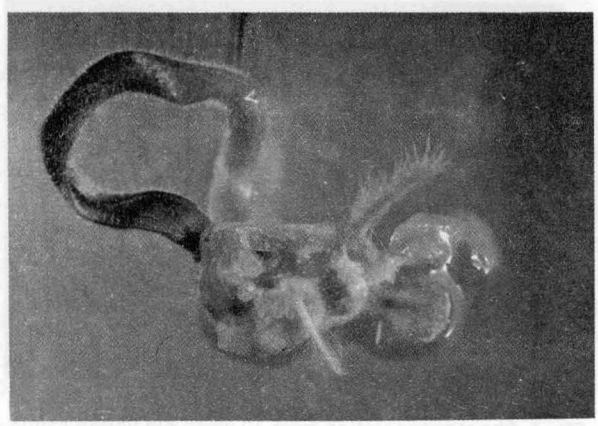

Fig. 3. Shoot regeneration from 6 month old callus of wheat

Role of glutamine

The role of glutamine in regeneration ability was studied in combination with cytokinin alone or with cytokinin and auxin (Fig. 4). The different media and genotypes showed significant differences. There was no regeneration when basal media did not contain growth regulators. The medium with 2 mg/l BAP showed an average of 21.3% regeneration frequency over the genotypes. However, when this medium was supplemented with 150 mg/l glutamine there was 6% increase in regeneration frequency. Medium with 3 mg/l BAP and BAP with glutamine showed an average regeneration frequency of 39.6 and 50% respectively. Thus, there was 10% increase in regeneration frequency by the presence of glutamine in the medium. In another combination where media contained cytokinin (3 mg/l) and auxin (0.5 mg/l) and when this was further supplemented with glutamine, again there was an increase of 10% in regeneration frequency. The regeneration frequency in media with cytokinin was 40%, cytokinin and auxin 45% while cytokinin, auxin and glutamine it was 55%. Thus, there was progressive increase in regeneration frequency.

The promotory effect of exogenous applied glutamine during organogenesis has been indicated [14, 15]. Higher regeneration from inflorescence derived calli has been reported when medium was supplemented with glutamine during callus induction [9]. The addition of asparagine also helps in increasing the regeneration ability [13]. In our studies the role of growth regulators and glutamine in enhancing regeneration frequency from old callus cultures has been amply demonstrated.

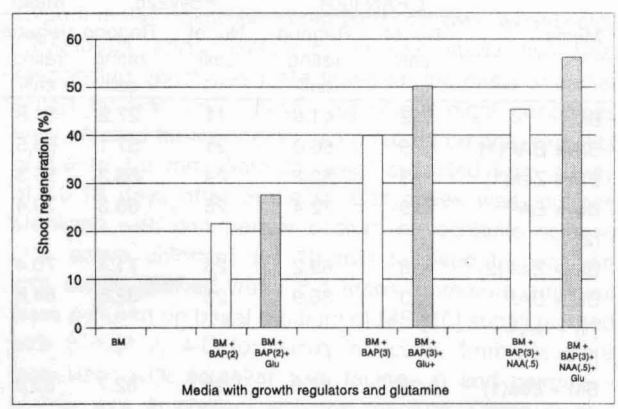

Fig. 4. The role of glutamine for shoot regeneration in immature embryo derived old callus cultures of wheat. (BM indicates basal medium of Murashige and Skoog and values in parentheses indicate concentration of growth regulators in mg/l)

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