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Full Length Research Paper

In vitro shoot regeneration from preconditioned explants of chickpea (*Cicer arietinum L.*) cv. Gokce

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The present study reports the successful shoot regeneration of preconditioned mature embryo and embryonic axis explants of chickpea cv. Gokce. Explants were preconditioned with 10 mgl benzylaminopurine (BA) for 7 days followed by culture on Murashige and Skoog (MS) medium containing 0.25, 0.50, 1.00 and 2.00 mg/l BA with or without 0.25 mg/l naphthalene acetic acid (NAA) supplemented with 4 mg/l activated charcoal and 1 mg/l polyvinylpyrrolidon (PVP). Shoot regeneration was recorded on all explants. Maximum number of (14.75) shoots per explants on mature embryo were recorded on MS medium containing 2.0 mg/l BA with 0.25 mg/l NAA. Whereas, 16.83 shoots per explants were recorded on MS medium containing 2.0 mg/l BA. Presence of NAA in the culture medium decreased the mean shoot length of both explants compared to medium devoid of 0.25 mg/l NAA. Regenerated shoots were rooted on MS medium containing 1.0 mg/l indole-butyric acid (IBA) after 4 weeks of culture. Rooted plantlets were transferred to pots for acclimatization under green house conditions.

Key words: *In vitro*, chickpea, pulse treatment, shoot regeneration.

INTRODUCTION

The chickpea (*Cicer arietinum L.*) is one of the earliest cultivated edible legumes used as vegetable. Chickpeas are grown in the Mediterranean, western Asia, the south Asia and Australia. They are used for make curries by cooking, making "*hummus",* by roasting and for making salads by using boiled chickpea seeds.

Modern biotechnology, including tissue culture, genetic engineering and genetic transformation techniques, has provided new opportunities to enhance the germplasm of crop plants (Sharma and Ortiz, 2000). However, a reliable shoot regeneration protocol is a prerequisite for efficient application of genetic transformation strategies (Jayanand et al., 2003). Shoot regeneration in chickpea by shoot organogenesis, somatic embryogenesis and callus using various explants has been reported previously (Polisetty et al., 1996, 1997; Paul et al., 2000; Rizvi and Singh, 2000; Khawar and Ozcan 2002, Chauhan et al., 2003; Jayanand et al., 2003; Sevimay et al. 2005, Chakraborti et al., 2006; Barna and Wakhlu, 1994; Sagare et al., 1993; Suhasini et al., 1994; Kumar et al., 1994; Kar et al., 1996, 1997; Rizvi and Singh, 2000; Chauhan and Singh, 2002; Kiran et al., 2005).

Pre-conditioning or pulse treatment of explants at higher concentration of cytokinin is often used for shoot regeneration (Brar et al., 1999; Aasim et al., 2009a, 2010). There is no report on the effects of preconditioning of chickpea explants with cytokinins on shoot regeneration. The study aimed to develop an efficient shoot regeneration system using pre-conditioned mature embryos and embryonic axis explants of chickpea cv. Gökce which is a newly developed highly drought tolerant cowpea specie of Turkey.

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Abbreviations: NAA, Naphthalene acetic acid; **MS,** Murashige and Skoog; **BA,** benzylaminopurine; **PVP,** polyvinylpyrrolidon; **IBA,** indole-butyric acid.

MATERIALS AND METHODS

The seeds of chickpea cv. Gokce were obtained from the Central Field Crops Research Institute, Ankara, Turkey. Mature seeds were first screened manually and mechanically damaged seeds were removed. Seeds were surface sterilized with 70% commercial bleach (ACE-Turkey) containing 5 - 6% NaOCl for 10 min followed by three times rinsing with bidistilled sterilized water for 5 min each to remove the traces of commercial bleach. Thereafter, the seeds were shaked in bidistilled sterilized water at 100 rpm for 24 h to soften and imbibe the seeds for easy recovery of embryos. .

After 24 h, mature embryo explants were isolated under *in vitro* conditions and conditioned on Murashige and Skoog (MS) (1962) medium containing 10 mg/l benzylaminopurine (BA) supplemented with 3% sucrose, and 6.5 g/l agar. After 7 days, the embryos and embryonic axis were isolated aseptically from preconditioned explants and were cultured on MS medium containing 0.25, 0.50, 1.00 and 2.00 mg/l BA with or without 0.25 mg/l naphthalene acetic acid (NAA). All culture media were supplemented with 3.0% sucrose, 6.5 g/l agar, 1.0 mg/l polyvinylpyrrolidon (PVP) and 4.0 mg/l activated charcoal.

The explants were subcultured to the same medium after 4 and 7 weeks on the same medium devoid of activated charcoal. After 10 weeks of culture, the regenerated shoots were rooted on MS medium containing 0.25, 0.50, 1.00 and 2.00 mg/l indole-butyric acid (IBA). After 4 weeks of rooting, the rooted plantlets were transferred to pots and acclimatized under growth room conditions.

All treatments of regeneration experiments had three replicates and repeated twice with 8 explants in each replication (3 replications x 8 explants x 2 repeats = 48 explants). Data for frequency (%) of shoot regeneration, mean number of shoots per explant, shoot length and frequency of rooting were recorded and analyzed using one way analysis (ANOVA) and the post hoc tests were performed using Duncans multiple range test with the help of statistical software Statistical Package for the Social Sciences (SPSS) 16.00 for windows. Data given in percentages were subjected to arcsine transformation (Snedecor and Cochran, 1967) before statistical analysis.

RESULTS

The present investigation showed the effect of preconditioning with BA on shoot regeneration from mature embryo and embryonic axis. Shoot regeneration was visible within two weeks on both explants on MS medium containing variants of BA-NAA. Direct shoot regeneration was recorded on mature embryos (Figure 1a) without callus induction on MS medium containing various concentrations of BA-NAA. Irrespective of the concentration of plant growth regulators, both direct and indirect shoot organogenesis was noticed on embryonic axis explants (Figure 1b). Explants were sub cultured to the same medium devoid of activated charcoal after 6 weeks which clearly enhanced the shoot regeneration and shoot length of both explants.

It was also observed that callus induction was more on MS medium containing BA-NAA compared to MS medium devoid of NAA (data not shown). However, callus induction did not affect the shoot regeneration frequency and 100% shoot regeneration frequency was recorded on both explants. Hyperhydric shoots with variable frequency were also observed on both explants. However, frequency of hyperhydric plants was subjected to explant type and culture medium. More hyperhydric plants were recorded on mature embryo explants compared to embryonic axis (data not shown). It was noticed that addition of NAA in the culture media resulted in inhibition of hyperhydricity.

Effect of medium composition on shoot induction

Although shoot regeneration was recorded on explants on all regeneration media, but mean number of shoots per explants were significantly different on both explants. Similarly, maximum number of 12.92 and 14.75 shoots per mature embryo explant was recorded on MS medium containing 2.00 mg/l BA with or without NAA, respectively (Table 1). However, 0.25 mg/l NAA with 0.25 BA was not favorable and resulted in minimum number of 8.42 shoots per mature embryo explant. Presence or absence of NAA affected the mean number of shoots per explants on embryonic axis. Increase in BA concentration without NAA increased mean number of shoots per explants with maximum of 16.83 shoots on MS medium containing 2.00 mg/l BA. Whereas, presence of NAA in the culture medium showed variable effect on shoot regeneration with 9.58 - 14.25 shoots per explants.

Effect of medium composition on shoot length

Both explants responded variably to the presence or absence of NAA in the culture medium. Presence of NAA in the culture medium had negative effect on shoot length and resulted in smaller shoots on both explants compared to MS medium without NAA. Relatively smaller shoots of 1.22 cm were recorded on mature embryo explants cultured on MS medium containing 0.50 mg/l BA without NAA (Table 1). Contrarily, longer shoots (1.95 cm) were recorded from embryonic axis on the same culture medium. Each increase in BA concentration with NAA decreased the mean shoot length on mature embryo explants compared to embryonic axis explants.

Rooting

About 1 cm long regenerated shoots from both explants were rooted on 1.0 mg/l IBA to achieve whole plant regeneration. Frequency (%) of rooting of both explants was 22.22% after 4 weeks of culture. Rooted plants (Figure 1c) were transferred to pots and acclimatized under greenhouse conditions to maturity at room temperature.

DISCUSSION

Preconditioning of explants using cytokinins is used for rapid multiplication of cells at initial stage for enhancing shoot regeneration, and is previously reported by Brar et

Table 1. Effect of pre-conditioning on number of shoots and shoot length of mature embryo and embryonic axis explant of chickpea cv. Gokce.

BA (mg/l)	NAA (mg/l)	Shoots per explants		Mean shoot length (cm)	
		Mature embryo	Embryonic axis	Mature embryo	Embryonic axis
0.25	-	11.83c	10.83c	1.56a	1.42bc
0.50	-	11.33c	10.50c	1.22b	1.95a
1.00	-	11.83c	14.17b	1.38ab	1.61ab
2.00	-	12.92b	16.83a	1.58a	1.45bc
0.25	0.25	8.42d	15.58ab	0.92c	0.72d
0.50	0.25	10.75c	9.58c	0.85cd	0.68d
1.00	0.25	10.42c	14.00b	0.63d	0.99cd
2.00	0.25	14.75a	14.25b	0.70cd	1.01cd

Mean values within a column followed by different letters are significantly different at the 0.05 probability level using Duncan's multiple range test.

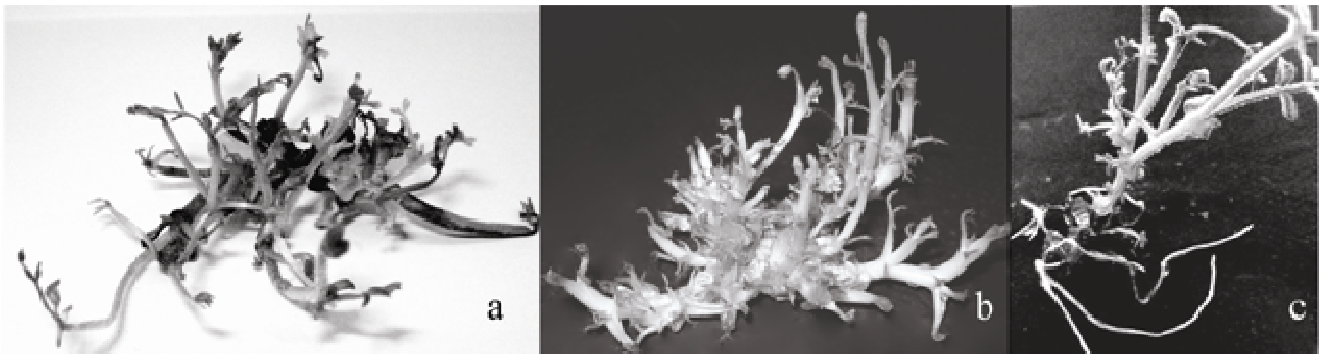

Figure 1. *In vitro* shoot regeneration of chickpea cv. Gokce on mature embryo (a), embryonic axis preconditioned with 10 mg/l BA for 1 week (b) and rooting of *in vitro* regenerated shoots on MS medium containing 1 mg/l IBA plantlet with root (c).

al. (1999) and Aasim et al. (2009a, 2010) in cowpea. Results showed that preconditioning of explants had positive effects on shoot regeneration frequency in line with Aasim et al. (2009a, 2010) who recorded 100% shoot regeneration in cowpea using preconditioned plumular apices and embryonic axis respectively.

Results showed variable shoot regeneration behavior of two explants in line with Polisetty et al. (1996, 1997), Paul et al. (2000), Rizvi and Singh (2000), Chauhan et al. (2003), Jayanand et al. (2003) and Chakraborti et al. (2006). NAA free medium did not induce callus, however, callus induction was recorded on explants cultured on MS medium containing NAA in agreement with Aasim et al. (2008) who reported that addition of NAA resulted in callus induction, which increased with the increase of concentration of NAA in cowpea.

Preconditioning of explants affected the extent of hyperhydricity. BA increase hyperhydricity was recorded on mature embryos compared to embryonic axis. However, a comparison of results showed that hyperhydricity was relatively high on both explants cultured on MS media supplemented with DL-β-aminobutyric acid (BABA) ≥ 1.00 BA with or without NAA. It was assumed that hyperhydricity might be due to preconditioning of explants with BA at initial stage followed by culturing on MS medium free of NAA in agreement with Ziv (1991) who reported that ethylene accumulation and dose of cytokinins caused hyperhydricity. Inclusion of auxin in the culture medium decreased the hyperhydricity in line with Aasim et al. (2009b), who reported positive effects of IBA on decreasing hyperhydricity in fenugreek. Contrarily, Aasim et al. (2009a) reported no hyperhydricity due to preconditioning of explants in cowpea, another legume.

Results on shoot length showed variable response of explants to culture medium. Inclusion of NAA in the culture medium decreased the mean shoot length on both explants. These results are contradictory to the findings of Aasim et al. (2008, 2009a) who reported positive effects of NAA in the culture medium on shoot length of cowpea.

Rooting experiments showed poor response of regenerated shoots to IBA with maximum of 22.22% root induction on 1.00 mg/l IBA. Krishnamurty et al. (2000) reported low root formation from excised shoots of chickpea. Roy et al. (2001) failed to get root formation from shoot regeneration obtained from chickpea callus in B5 medium

containing NAA and BA. However, Polisetty et al. (1996, 1997) and Sanyal et al. (2005) reported high percentage of root induction from excised shoots in a medium containing reduced nitrogen and sucrose. Rooted plants that were transferred to pots for acclimatization under greenhouse conditions showed poor response and the plants failed to survive. Contrarily, Chakraborty et al. (2006) grafted shoots on root stocks and obtained 90 - 95% survival in soil after hardening. Khawar and Ozcan (2004) used *Agrobacterium rhizogenes* for root formation in chickpea. There is need to carry out more work on rooting and acclimatization to solve the problem of this important legume crop.

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