Studies on In Vitro Regeneration of Some Common Bean (Phaseolus vulgaris L.) Cultivars

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Abstract: The propagation possibilities of different commercial common bean cultivars (*Phaseolus vulgaris*) by tissue culture were investigated in the research. For this purpose, Yörük Ayşe, Alman Ayşe 4, Alman Ayşe 5, Alman Ayşe 6 and Alman Ayşe 7 cultivars were used as plant materials. On the condition of tissue culture shoot tips were used for shoot formation (%) and hypocotyls were used for root formation (%) as explant materials. The highest shoot evolution was obtained from MS0 for Yörük Ayşe, for MS2 (1ml/l NAA + 1ml/l BAP + 1ml/l GA_3) for Alman Ayşe 4 and Alman Ayşe 7 cultivars, from MS3 (2ml/l NAA + 2ml/l BAP + 2ml/l GA_3) for Alman Ayşe 6. In terms of root formation the highest root formation was obtained from MS4 (MS0 + 3g/l active charcoal) in Alman Ayşe 4 with the proportion of %92.

Keywords: Common bean, *Phaseolus vulgaris* L., *in vitro*, regeneration

Introduction

Phaseolus vulgaris L. (common bean) is an important member of genus Phaseolus and 90% of cultivated bean comes from Phaseolus vulgaris L. Common bean can be grown in all continents, except Antarctica and is the most important grain legume for human consumption specaily for meeting the need of protein. (Singh, 1999; Larsen, 2005). Due to easy growing and its importance in human nutrition common bean growing has become very popular in Turkey and all around the world and that is why many physiological and biochemical experiments have been conducted on bean (Torres et al., 2004).

Classical breeding is the basic and general approach for production of the widespread varieties. Some problems such as genetic variations, low surviving ability of the interspecific hybrids, specific inheritances of some valuable characteristics, yield, disease and pests' resistance, etc., are somehow difficult or time and labor consuming to be resolved by the conventional techniques. Plant biotechnology offers different strategies to overcome these difficulties (Veltcheva and Svetleva, 2005).

With some exceptions and generally speaking species in the family Leguminosae are difficult to regenerate in in vitro conditions as grain legumes have less regeneration potential compared to some others. It is possible to say that regeneration ability depends on the genotype, physiological state of the explant and donor plant, tissue and cell specialization of the culture and the culture conditions (Veltcheva and Svetleva, 2005). In accordance with the previous studies there is no great success in the studies conducted on in vitro regeneration of common bean in Turkey (Sağlam et al., 2005). The present study is conducted to reveal the propagation possibilities of different commercially grown bean cultivars (Phaseolus vulgaris L.) in vitro conditions by using shoot tips and hypocotyls as explants.

Material And Methods

Yörük Ayşe, Alman Ayşe 4, Alman Ayşe 5, Alman Ayşe 6 and Alman Ayşe 7 cultivars, which are commonly grown and have commercial importance, were used as plant materials.

Fully mature seeds of any cultivar were imbedded in distilled water prior to sterilization in order to simplify the process of removing testa from endosperm. All the seeds were subjected to surface sterilization by

keeping them in 5% sodium hypochlorite for 10 minutes. After 10 minutes seeds were transferred into a solution of 70% ethanol and kept for 2 minutes, then they were rinsed three times for 5 minutes each time in sterile distilled water and kept in the last wash.

Each endosperm-embryo complex was put on the surface of 2 culture media which consisted of 5 ml. Murashige & Skoog's medium (MS) (Murashige and Skoog, 1962) with an agar concentration of 0.7% w/v, pH 5.8 in order to determine the best medium for seed germination. It should be noted that while one of the MS medium was free of all plant growth regulation (MS0), 1 ml/l GA_3 was added to other MS medium. All the experiments were set up as three replicates with 2 phials in each replication and 5 seeds in each phial. The culture phials were placed in constant temperature room (25±3 °C) until the seedlings had developed hypocotyls.

Hypocotyls and shoot tips of in vitro grown seedlings were excised and used as explants. The culture phials were placed in a constant temperature room (25±3 °C) to make the observations. In order to determine the regeneration capacities of these two different kinds of explants different culture media with different contents were used as stated in Table 1.

Media used for shoot tip explants

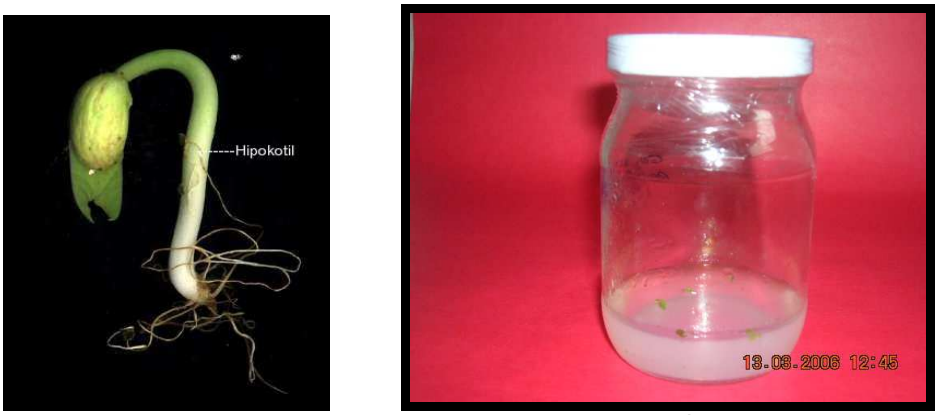
MS1	MS0
MS2	MS0 + 1 ml/l NAA + 1 ml/l BAP + 1 ml/l GA_3
MS3	MS0 + 2 ml/l NAA + 2 ml/l BAP + 2 ml/l GA_3

Media used for hypocotyls explants

MS4	MS0 + 3 g/l active charcoal
MS5	MS0 + 1 ml/l NAA + 1 ml/l BAP + 1 ml/l GA_3 + 3 g/l active charcoal
MS6	MS0 + 2 ml/l NAA + 2 ml/l BAP + 2 ml/L GA_3 + 3 g/l active charcoal

Table 1. Culture media

Five shoot tip explants and five hypocotyls explants were taken and placed into phials. Hypocotyls explants were taken from the part close to roots assuming the cells in the part have the more ability for rooting than the other parts (Figure 1).



a b

Figure 1. The part of plant where hypocotyls explants were taken (a), shoot tips explants in a phial (b)

Afterwards responds of all cultivars in different culture media were recorded and percentages of shoot formation and root formation calculated as down stated.

$$\%\ \text{Shoot formation} = \frac{\text{Number of shoot tips forming shoots}}{\text{Total number of shoots}} \times 100$$

$$\%\ \text{Root formation} = \frac{\text{Number of hypocotyls forming roots}}{\text{Total number of roots}} \times 100$$

RESULTS AND DISCUSSION

Experiment results conducted in order to determine the best medium for seed germination revealed that there is no need to add 1 ml/l GA_3 into the media as all the seeds (a total of 30 seeds) in both media were

germinated at the end of 10 days period (Figure 2).

Figure 2. Seedlings developed in MS0 media at the end of 10 days period

Hypocotyls and shoot tips of in vitro grown seedlings were excised and used as explants in order to determine the regeneration capacities of these two different kinds of explants and different culture media with different contents were used to serve the purpose. Experiment results revealed that shoot tips explants formed leaf and shoot elongation took a part in 8-10 days period (Figure 3). On the other hand hypocotyls explants resulted with root formation with no shoot (Figure 3-b).

a

b

Figure 3. Shoot formations from shoot tip explants (a) and root formation from hypocotyls explants (b)

Afterwards responds of all cultivars in different culture media were recorded and percentages of shoot formation and root formation for each cultivar were calculated and results are presented in Table 2 and Table 3.

	MS2	10	83
Cultivar	Culture media	Total number of shoots obtained	% shoot formation
Yörük Ayşe	MS1	12	100
	MS2	10	83
	MS3	9	75
Alman Ayşe 4	MS1	7	58
	MS2	12	100
	MS3	8	67
Alman Ayşe 5	MS1	12	100
	MS2	10	83
	MS3	8	67
Alman Ayşe 6	MS1	8	67
	MS2	7	58
	MS3	12	100
	MS1	9	75

Table 2. Shoot formation obtained from shoot tip explants

As can be seen from Table 2, MS1 media was the most suitable medium for Yörük Ayşe and Alman Ayşe 5 cultivars. MS2 for Alman Ayşe 4 and Alman Ayşe 7 and MS3 for Alman Ayşe 6 media were found to be the most suitable for culture media were shoot formation. Kartha et al. (1981) reported that culture media with different concentrations of BA gave the best result on in vitro regeneration of bean. Cruz de Carvalho et al. (2000) reported that 10µM N^6 -benzylaminopurine (BAP) and 10µM silver nitrate ($AgNO_3$) increased the level of shoot development in vitro development of common bean. Results obtained from the present study are,therefore, in agreement with previous findings as culture media needs an effective cytokinin source for process of shoot initiation and elongation as reported by Veltcheva et al. (2005).

Cultivar	Culture media	Total number of explants formed roots	% root formation
Yörük Ayşe	MS4	5	42
	MS5	6	50
	MS6	4	33
Alman Ayşe 4	MS4	11	92
	MS5	6	50
	MS6	7	58
Alman Ayşe 5	MS4	2	17
	MS5	2	17
	MS6	2	17
Alman Ayşe 6	MS4	6	50
	MS5	6	50
	MS6	0	0
Alman Ayşe 7	MS4	8	67
	MS5	5	42
	MS6	0	0

Table 3. Root formation obtained from hypocotyls explants

As can be seen from Table 3 the highest root formation (92%) was obtained for Alman Ayşe 4 cultivar in MS4 culture media. For all cultivars the highest and lowest root formations were obtained from MS4 and MS6 culture media respectively. Results obtained in this present study are in agreement with Adak et al. (2001) as they reported that active charcoal had a positive impact on in vitro root formation and development in strawberry.

As a conclusion; simple MS (MS0) is good enough culture media for seed germination of common bean. Callus formation took place from hypocotyls explants and root formation occurred in active charcoal added culture media. On the other hand when shoot tips were used as explants for shoot formation occurred with different cultivars in different culture media. As no culture media was found to be suitable for both shoot and root formation further studies are necessary to be conducted to find the optimum media.

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