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RESEARCH ARTICLE

IN VITRO PROPAGATION OF WHITE LUPIN (*LUPINUS ALBUS* L.) FROM SHOOT TIP EXPLANTS

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ABSTRACT: *Lupinus albus*, commonly known as white lupin, is used as food, medicine and to maintain soil fertility. Low yield and high alkaloid content are among the major constraints of this crop. Plant tissue culture that usually starts with *in vitro* propagation as pre-requisite can be used for crop improvement. Therefore, the objective of this study was to develop *in vitro* propagation protocol for this crop. The seeds were sterilized with sodium hypochlorite and cultured on growth regulators free MS medium. Shoot tips from *in vitro* germinated seedlings were cultured on MS medium supplemented with 0.5 and 1.0 mg/l BAP or kinetin alone. The shoots were cultured on half strength MS medium containing BAP (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) in combination with kinetin (0.0, 0.5, 1.0, and 1.5 mg/l) and NAA (0.0, 0.01, 0.1, 0.5 and1.0 mg/l) for shoot multiplication. The multiplied shoots were cultured on half strength MS medium supplemented with 0.01 mg/l IBA, 0.1 mg/l IAA or 0.5 mg/l NAA for rooting. Seeds sterilized with 1.5% sodium hypochlorite for 20 min showed 100% germination. The highest mean shoot number per explant (7.53±0.64) was obtained on medium supplemented with 2.0 mg/l BAP in combination with 0.5 mg/l kinetin. The highest mean root number per shoot (18.36±1.28) and mean root length (3.43 ±0.13 cm) was obtained on half strength MS medium supplemented with 0.01 mg/l NAA. After one month of acclimatization, 90% of the plants survived. This protocol can be used` for genetic improvement of *Lupinus albus* using biotechnological approaches.

Key words/phrases: Plant growth regulators, Seed germination, Shoot multiplication, Shoot tip.

INTRODUCTION

The genus *Lupinus albus* L. commonly known as white lupin belongs to the family Fabaceae (Gladstones, 1998). It is one of the most species-rich, diverse and widespread legume genus encompassing 200 to 500 species originating from both Old and New World (Mihailović *et al*., 2007; Wolko *et al*., 2011). The genus includes both annual and perennial herbaceous

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species, a few soft-woody shrubs and small trees, which occur in a wide range of eco-geographical conditions. The most important crops in this genus are white (*L. albus* L.), yellow (*L. luteus* L.), narrow-leafed (*L. angustifolius* L.) and Andean (*L. mutabilis*) lupins (Dunn, 1984; Turner, 1995).

White lupin, locally known as 'Gibto' in Ethiopia, is an attractive grain legume containing high protein content (34–45%), similar to that of soybean. Low content of anti-nutritional factors and alkaloids in lupines is suggested as an alternative to soybean for producing local foods and also for beverages and protein products (Sator, 1982). The essential amino acids as well as iron and potassium content of white lupin is higher than pea, faba bean and narrow leafed lupin (Sulas *et al*., 2016).

White lupins are important crop grown worldwide as a source of protein both for human food and animal feed. The culture of lupin is vital to overcome the problem of protein deficiency in animal nutrition. In addition, lupin fertilizes the soil by fixing the atmospheric nitrogen and improves the structure and porosity of the soil. Consequently, it is ideal for inclusion in crop rotation such as with cereals (Sator, 1985). This crop should be considered as a model plant of P-efficient and low input species, especially on marginal lands (Hondelmann, 1984).

In Ethiopia, the farmers use their own traditional production system. Lack of production packages, late maturity and diseases are the major constraints in production and utilization of lupin. In addition, it contains undesirable characters, such as high alkaloid level and is low yielder (Mulugeta Atnaf *et al*., 2020). Therefore, Hibstu Azeze *et al*. (2016) recommended that the research system should work on white lupin to generate improved production and utilization technologies.

There is an increasing interest in breeding lupins because of its high protein and oil content (Lucas *et al*., 2015). Besides containing useful substances, *Lupinus albus* has also negative impacts such as lupinosis, a disease that primarily damages the liver, causes loss of appetite and sometimes may lead to death suggesting the need for improvement of this crop. This might be solved through genetic engineering or genome editing. The role of genetic engineering or genome editing is not only adding desirable traits to the crops but also removing unnecessary and harmful traits from the target crop.

To improve plants through genetic engineering or genome editing, different tissue culture protocols such as *in vitro* propagation and *in vitro*

regeneration are pre-requisite. *In vitro* techniques could be used to obtain somaclonal induced variation or they could be adopted for *Agrobacterium* mediated transformation to introduce insect, viral and herbicide resistance or to improve nutritional value. As Sator (1985) and Nadolska-Orczyk (1992) reported, most of the leguminous plants, *Lupinus* are very difficult material for tissue culture manipulation and transformation and limited successes were obtained in the *in vitro* propagation of several lupine species. It is therefore, important to develop an efficient and reproducible protocol for the *in vitro* propagation of *Lupinus* spp. Therefore, the objective of the present study was to develop an efficient *in vitro* propagation protocol for *Lupinus albus*.

MATERIALS AND METHODS

Plant materials

The seeds of *Lupinus albus* were bought from local market of Amanuel, north East Gojam, and Pikolo Abay, north west of Gojam. Damaged, infested or deformed seeds were discarded and healthy and uniform seeds were kept at room temperature.

Seed sterilization and germination

The seeds were washed under running tap water followed by washing with detergent and then rinsed with 70% ethanol for one min. After rinsing three times with sterile distilled water, the seeds were surface sterilized with 20% (1% active chlorine) and 30% (1.5% active chlorine) sodium hypochlorite for 15, 20 and 25 min. The seeds were then washed three times with sterile distilled water. For each sterilization treatment, six seeds per culture jar with five replications were used.

After surface sterilization, seeds were directly cultured in culture vessels containing 50 ml full strength MS medium (Murashige and Skoog, 1962) containing 3% sucrose and 7 g/l agar. The pH of the medium was adjusted to 5.8 before addition of agar and then autoclaved. The culture jars were properly sealed with parafilm and maintained under 16 h photoperiod and light intensity of 22 μmol $m^{-2}s^{-1}$ using cool-white fluorescent lamps at 25±2°C. Contamination free seeds and the number of germinated seeds were recorded after four days of culturing until no further germination occurred.

Shoot initiation

Shoots were excised from the seedlings consisting of 3–4 nodes and cultured in culture vessel containing 50 ml full strength MS medium

supplemented with 0.5 and 1.0 mg/l BAP and kinetin alone, 30 g/l sucrose and 7.0 g/l agar. Five explants per culture vessel and six replications per treatment were used. All cultures were maintained under 16 h photoperiod at light intensity of 22 μmol $m^{-2}s^{-1}$ provided with white fluorescent light at 25±2°C. Number of shoots and shoot length per explant were recorded after four weeks of culture.

Shoot multiplication

The initiated shoots from initiation medium were transferred to culture vessel containing 50 ml full strength MS medium supplemented with BAP (0.0, 0.5, 1.0, 1.5 and 2.0 mg/l) in combination with kinetin (0.0, 0.5, 1.0, and 1.5 mg/l) and α-naphthalene acetic acid (NAA) (0.0, 0.01, 0.1, 0.5 and 1.0 mg/l). Each culture vessel contained five explants and each experiment was done in six replications. The cultures were maintained at the same culture conditions as that of shoot initiation. After four weeks of culturing, shoot number and length per explant were recorded.

Rooting

For root induction, half strength MS medium supplemented with 0.0, 0.01, 0.1, 0.5 mg/l NAA alone, indole-acetic acid (IAA) alone and indole-3-butyric acid (IBA) alone were used. The pH was adjusted to 5.8 prior to autoclaving. Multiplied shoots from shoot multiplication medium were transferred to the rooting media. Five explants per culture vessel were used and each treatment was replicated six times. The cultures were maintained at culture conditions as that of shoot initiation. Root number and length per explant were recorded after two weeks.

Acclimatization

Well rooted plantlets were gently removed from the rooting media and the roots were washed under running tap water. The plantlets were planted in plastic pots containing sterile garden soil. The pots were covered with aerated polyethylene bag and kept in the culture room for two weeks and watered at an interval of two to three days. After two weeks, the plants were shifted to greenhouse and covered with light polyethylene bags. The polyethylene bags were gradually removed after two weeks. The number of survived plants was recorded after one month.

Data analyses

Completely Randomized Design (CRD) was used and for shoot initiation, multiplication and rooting, five explants per culture vessel in six replications

were used. Data were subjected to analysis of variance (ANOVA) and least significant difference (LSD) test using statistical data analysis software SPSS 20.0 version at 0.05 probability level.

RESULTS

Seed sterilization and germination

Among the different concentrations of sodium hypochlorite used, 30% (1.5% active chlorine) with 20 min exposure time and 20% (1% active chlorine) with 25 min exposure time resulted in the highest (100%) contamination free seeds (Table 1). The highest contamination (100%) of seeds was recorded from control treatment which contained seeds cultured without sodium hypochlorite treatment followed by 56% and 37.3% contamination recorded from 20% (1% active chlorine) sodium hypochlorite with exposure time of 15 min and 20 min, respectively. Least germination percentage (43.3%) was obtained from 20% (1% active chlorine) sodium hypochlorite with exposure time of 15 min and 54.2% from 30% (1.5% active chlorine) sodium hypochlorite solution with 25 min exposure time (Table 1).

Disinfection of *L. albus* seeds with 20% (1% active chlorine) and 30% (1.5% active chlorine) sodium hypochlorite for 25 and 20 min exposure time were the most effective and recommendable sterilization treatments. These sterilization treatments resulted in the highest germination percentage (100% and 96.7%, respectively) with 100% contamination free explants.

Table 1. Effect of different concentrations of sodium hypochlorite and exposure time on *in vitro* germination of *Lupinus albus* seeds.

Sodium hypochlorite (%)	Exposure time (min)	Clean explants (%)	Contamination (%)	Germination (%)
0.0	20	00	100	60
20	15	46	56	63.3
20	20	63.7	37.3	70
20	25	100	00	96.7
30	15	76.7	23.3	100
30	20	100	00	100
30	25	100	00	54.2

Shoot initiation

Significant difference ($P<0.05$) in shoot initiation, the mean number of shoots per explant and shoot length was observed on MS medium supplemented with 0.5 and 1.0 mg/l BAP and kinetin. Relatively high concentration of BAP or kinetin (1.0 mg/l and above) was found to be better than low concentration (0.5 mg/l) for shoot initiation (Table 2). The

percentage of shoot initiation ranged from 53% to 100%. MS medium supplemented with 1.0 mg/l BAP resulted in 100% shoot initiation (Fig. 1A) followed by medium containing 1.0 mg/l kinetin (86.6%) and 0.5 mg/l BAP (80%). On the other hand, the lowest shoot initiation (53.33%) was observed on medium containing 0.5 mg/l kinetin.

Result of mean shoot number and shoot length are tabulated in Table 2. The highest mean shoot number per explant (3.73±0.23) was obtained on MS medium containing 1.0 mg/l BAP followed by 3.10±0.27 on MS medium containing 1.0 mg/l kinetin. Although there was no significant difference in mean shoot number per explant among the media containing 0.5 mg/l BAP, 0.5 mg/l kinetin and the control, the lowest mean shoot number per explant (1.73±0.17) was obtained on growth regulators free MS medium, which was used as a control. The highest mean shoot length per explant (3.22±0.13 cm) was obtained from medium containing 1.0 mg/l kinetin and the lowest mean shoot length (1.73±0.13 cm) was obtained on medium containing 0.5 mg/l BAP (Table 2). However, there was no significant difference between medium containing 1.0 mg/l BAP and 0.5 mg/l kinetin on mean shoot length.

Table 2. Effect of BAP and kinetin on shoot initiation of *Lupinus albus*, values are given as mean ± SE.

BAP (mg/l)	Kinetin (mg/l)	Shoot initiation (%)	No. of shoots/ explant	Shoot length (cm)
0.00	0.00	63.3	1.73 ± 0.17^{c}	1.52 ± 0.18^{bc}
0.5	0.00	80	2.26 ± 0.19^{bc}	1.17 ± 0.13^{c}
1.0	0.00	100	3.73 ± 0.23^{a}	1.77 ± 0.09^{b}
0.0	0.5	53	1.83 ± 0.20^{c}	1.73 ± 0.13^{b}
0.0	1.0	86.6	3.10 ± 0.27^{ab}	3.22 ± 0.13^{a}

Means followed by the same letter within a column are not significantly different at 5% probability level.

Shoot multiplication

Effect of BAP and kinetin on shoot multiplication

The result of the effect of BAP and Kinetin on shoot multiplication are tabulated in Table 3. The highest mean shoot number per explant (7.53±0.64) was obtained on medium containing 2.0 mg/l BAP (Fig. 1B) followed by medium containing 2.0 mg/l BAP in combination with 0.5 mg/l kinetin that produced 7.43±0.47 mean shoot number per explant (Table 3). The highest mean shoot length (4.11±0.23 cm) was obtained on medium supplemented with 0.5 mg/l BAP in combination with 1.5 mg/l kinetin and 3.97±0.32 cm was obtained on medium containing 1.0 mg/l BAP in combination with 1.5 mg/l kinetin.

Table 3. Effect of different concentrations of BAP in combination with kinetin on shoot multiplication of *Lupinus albus*, values are given as mean ± SE.

BAP (mg/l)	Kinetin (mg/l)	No. of shoots/ explant	Shoot length (cm)
0.0	0.0	2.26±0.23^{h}	3.97±0.32^{ab}
0.0	0.5	3.80±0.26^{defgh}	2.14±0.11^{de}
0.0	1.0	3.26±0.31^{gh}	1.84±0.14^{de}
0.0	1.5	2.20±0.22^{h}	2.68±0.17^{ce}
0.5	0.0	4.70±0.32^{cefg}	2.38±0.12^{de}
1.0	0.0	5.16±0.50^{bcdefg}	2.40±0.14^{de}
1.5	0.0	5.56±0.40^{abcdef}	2.13±0.14^{de}
2.0	0.0	7.53±0.64^{a}	1.53±0.18^{e}
0.5	0.5	4.20±0.43^{cdefgh}	2.81±0.17^{bcd}
0.5	1.0	3.93±0.32^{defgh}	2.97±0.21^{abcd}
0.5	1.5	3.51±0.22^{fgh}	4.11±0.23^{a}
1.0	0.5	5.60±0.35^{abcde}	1.99±0.19^{de}
1.0	1.0	3.36±0.30^{gh}	2.84±0.23^{bcd}
1.0	1.5	3.66±0.34^{efgh}	2.38±0.25^{de}
1.5	0.5	6.07±0.56^{abc}	2.52±0.19^{de}
1.5	1.0	5.83±0.36^{abcd}	3.73±0.20^{abc}
1.5	1.5	4.56±0.51^{cdefg}	2.52±0.18^{de}
2.0	0.5	7.43±0.47^{a}	2.62±0.54^{cde}
2.0	1.0	6.96±0.58^{ab}	2.58±0.32^{cde}
2.0	1.5	5.60±0.45^{abcde}	1.88±0.18^{de}

Means followed by the same letter within a column are not significantly different at 5% probability level.

Effect of BAP and NAA on shoot multiplication

MS medium containing different concentrations of BAP in combination with NAA produced shoots that were significantly different at P≥0.05 (Table 4). The highest mean shoot number per explant (3.30±0.36) was obtained on MS medium supplemented with 1.5 mg/l BAP in combination with 0.1 mg/l NAA. Although there was no significant difference, the highest mean shoot length per explant (1.90±0.15 cm) was obtained on MS medium supplemented with 1.5 mg/l BAP in combination with 0.5 mg/l NAA. The least mean shoot number per explant (1.70±0.20, 1.80±0.28 and 1.90±0.25) was recorded on MS medium supplemented with 0.5 mg/l NAA in combination with 0.5 mg/l BAP, 0.1 mg/l NAA in combination with 0.5 mg/l BAP and 0.1 mg/l NAA in combination with 1.0 mg/l BAP. There was no significant difference on shoot length among shoots obtained on media containing different concentrations of BAP in combination with NAA. However, relatively the highest mean shoot length (1.90±0.15 cm) was obtained on medium containing 0.5 mg/l NAA in combination with 1.5 mg/l BAP. The least mean shoot length per explant (1.24±0.58 cm) was obtained on medium containing 0.5 mg/l BAP (Table 4).

Table 4. Effect of different concentrations of BAP in combination with NAA on shoot multiplication of *Lupinus albus*, values are given as mean ± SE.

NAA (mg/l)	BAP (mg/l)	No. of shoots/ explant	Shoot length (cm)
0.0	0.0	2.26±0.23^{h}	3.97±0.32^{ab}
0.01	0.5	2.06±0.26^{ab}	1.62±0.08^{a}
0.01	1.0	2.66±0.25^{ab}	1.67±0.13^{a}
0.01	1.5	2.90±0.32^{ab}	1.33±0.16^{a}
0.1	0.5	1.80±0.28^{b}	1.56±0.16^{a}
0.1	1.0	2.76±0.37^{ab}	1.47±0.19^{a}
0.1	1.5	3.30±0.36^{a}	1.78±0.16^{a}
0.5	0.5	1.70±0.20^{b}	1.24±0.58^{a}
0.5	1.0	1.9000±0.25^{b}	1.31±0.16^{a}
0.5	1.5	2.06±0.24^{ab}	1.90±0.15^{a}
1.0	0.5	2.20±0.22^{ab}	1.33±0.16^{a}
1.0	1.0	2.30±0.24^{ab}	1.37±0.17^{a}
1.0	1.5	2.36±0.20^{ab}	1.72±0.118^{a}

Means followed by the same letter within a column are not significantly different at 5% probability level.

Rooting and acclimatization

Effect of NAA, IAA and IBA on rooting

All shoots cultured on rooting media produced roots (Fig. 1C). The highest mean number of roots per shoot (18.36±1.28) was obtained on 1/2 strength MS medium containing 0.1 mg/l NAA followed by 0.5 mg/l NAA (9.96±0.79) (Table 5). Shoots cultured on half strength MS medium supplemented with 0.01 mg/l IAA and the control produced the lowest mean root number per explant (1.33±0.28) and (1.40±0.26), respectively. The highest mean root length per explant (3.43±0.13 cm) was obtained on growth regulators free half strength MS medium which was used as control. After one month of acclimatization, 90% of the plants survived in the greenhouse (Fig. 1D).

Table 5. Mean number and length of roots obtained on half strength MS medium containing different concentrations of IBA, NAA and IAA, values are given as mean ± SE.

NAA (mg/l)	IBA (mg/l)	IAA (mg/l)	No. of roots/explants	Root length (cm)
00	00	00	1.40±0.26^{d}	1.78±0.08^{c}
0.01	00	00	2.83±0.43^{cd}	2.95±0.22^{a}
0.1	00	00	18.36±1.28^{a}	3.43±0.13^{a}
0.5	00	00	9.96±0.79^{b}	1.72±0.09^{c}
00	0.01	00	1.70±0.32^{d}	1.26±0.04^{c}
00	0.1	00	3.10±0.42^{cd}	1.54±0.07^{c}
00	0.5	00	4.93±0.60^{c}	2.37±0.16^{b}
00	00	0.01	1.33±0.28^{d}	1.30±0.05^{c}
00	00	0.1	2.83±0.46^{cd}	1.46±0.08^{c}
00	00	0.5	3.76±0.62^{cd}	1.49±0.08^{c}

Means followed by the same letter within a column are not significantly different at 5% probability level.

Fig. 1. *In vitro* propagation of *Lupinus albus*. (A) Shoot initiation on MS medium containing 1.0 mg/l BAP, (B) Shoot multiplication on MS medium containing 2.0 mg/l BAP, (C) Root formation on half strength MS medium containing 0.1 mg/l IBA, and (D) After one month of acclimatization in greenhouse.

DISCUSSION

Seed sterilization and germination

The present study demonstrated the effect of different concentrations of sodium hypochlorite and exposure time on lupin seeds. Analysis of variance (ANOVA) indicated that concentration of sodium hypochlorite, exposure time, and their interaction had highly significant difference ($P>0.05$) on avoiding contamination and improving survival and germination percentage of *L. albus in vitro* seed culture. From this study, among the different concentrations of sodium hypochlorite and different exposure time, 30% (1.5% active chlorine) sodium hypochlorite treatment with exposure time of explants to the sterilant for 20 min resulted in 100% contamination-free explants with 100% seed germination. Esmail *et al*. (2018) surface sterilized

seeds of *Lupinus termis* L. with 50% (2.5% active chlorine) sodium hypochlorite for 15 minutes.

The second seed germination percentage (86.7%) was obtained at 20% (1% active chlorine) sodium hypochlorite concentration and 25 min exposure with 100% contamination-free explants. This shows that the concentrations of active sodium hypochlorite solution and exposure time are interrelated factors to obtain contamination-free explants. Similarly, Sen *et al*. (2013) reported that higher concentration of sterilizing agents including NaOCl showed maximum effect against microbial contamination on *Achyranthes aspera*, but the survival percentage was low. This could be due to the fact that at high concentration of the sterilant, the damage resistance of plants may be different. When sodium hypochlorite concentration decreased, the seeds decayed and became contaminated.

Poor germination (54.2%) was recorded from 30% (1.5% active chlorine) sodium hypochlorite with exposure time of 25 min. With increasing concentration of sterilant and exposure time, rate of germination decreased. Supportive results were reported by Maina *et al*. (2010) that further increase in exposure time led to a significant decline in the survival rate of groundnut. Tola Bayisa *et al*. (2011) also reported that sterilants were toxic to the plant tissues; hence proper concentration of sterilants and exposure time of the explants to the sterilants, the sequences of using these sterilants should be standardized to minimize explant damage and achieve better survival.

Without sodium hypochlorite treatment, germination percentage of *L. albus* seeds was 60% with final 100% contamination. Sodium hypochlorite is a potent disinfecting agent which is widely used for seed surface sterilization (Kaneko and Morohashi, 2003). It is also known to favour seed germination or to overcome seed dormancy of different species (Vujanovic *et al*., 2000; Ervin and Wetzel, 2002).

Shoot initiation

Shoots cultured on control and MS medium supplemented with 0.5 and 1.0 mg/l BAP, and kinetin separately showed significant variation ($P<0.05$). Proliferation of shoots at 1.0 mg/l BAP resulted in 100% shoot initiation. Similarly, Karim *et al*. (2002) reported that 1.0 mg/l BAP resulted in maximum shoot initiation in chrysanthemum using shoot tips as explant. Ali *et al*. (2008) also reported that 1.0 mg/l BAP resulted in the highest number of shoots in carnation. Previous researchers have also confirmed that BAP

accelerates the development of bud initiation causing the increased number of buds primordial in chrysanthemum (Chagas *et al*., 2004; Aftab *et al*., 2008). The differences on shoot initiation percentage of BAP and kinetin could be a consequence of their differential uptake and translocation rates. Ugandhar *et al*. (2012) indicated that maximum number of shoots was induced on MS medium supplemented with various concentrations of BAP and kinetin. Due to their ability of enhancing shoot initiation, cytokinins are key factors for multiple shoot proliferation. Although the objective was to initiate shoots, multiple shoots could be obtained during shoot initiation stage in the present study. Accordingly, maximum shoot induction was obtained on MS medium containing 1.0 mg/l of both BAP and kinetin. The shoot initiation percentage was greatly influenced by the type and concentrations of cytokinin, which is in agreement with Arya *et al*. (2012).

Shoot multiplication

MS medium supplemented with BAP alone showed higher number of shoots per explant than combination of BAP with NAA, and kinetin indicating that BAP is more effective than kinetin. BAP is commonly used for *in vitro* culture of large-seed legumes. Sunder *et al*. (1989) reported BAP was proved to be ideal for shoot multiplication of shoot tip culture in grain legumes. In the present study, the highest mean shoot number (7.53±0.64) was obtained on MS medium containing 2.0 mg/l BAP. Li *et al*. (2000) also used 2.0 mg/l BAP that resulted in the highest shoot number in *Lupines luteus*. Ye *et al*. (2002) also reported the same result on lentil (*Lens culinaris*). When BAP and kinetin concentrations were increased beyond 2.0 mg/l, the rate of shoot multiplication and elongation was reduced due to supra-optimal concentration as also reported by Ugandhar *et al*. (2012). In contrast, Pniewski *et al*. (2002) reported the requirement of low concentration of BAP (0.05–0.25 mg/l) for *L. albus* shoot multiplication. However, better result was obtained on MS medium containing 2.0 mg/l BAP in the present study. This difference could be due to the difference in genotypes of *L. albus*. The effectiveness of BAP may lie on its ability to enhance the production of natural endogenous hormones inducing shoot organogenesis. That is the reason why BAP is the most widely used and effective cytokinin for various legumes (Saini and Jaiwal, 2002).

When the concentration of BAP and kinetin was increased beyond 2.0 mg/l, the rate of shoot multiplication and elongation was reduced in the present study. MS medium supplemented with 1.0 mg/l kinetin was not favourable for shoot multiplication in chickpea (*Cicer arietinum* L.) (Kadiri *et al*.,

2014). Among different concentrations of BAP combined with NAA, medium containing 1.5 mg/l BAP in combination with 0.1 mg/l NAA resulted in the highest mean shoot number per explant (3.30±0.36). Griga *et al*. (1986) obtained multiple shoots on MS medium supplemented with 1.5 mg/l BAP in combination with 0.1 mg/l NAA in pea. Both BAP and NAA are important plant growth regulators used by a number of researchers for shoot regeneration in a number of plant species such as grass pea (Barpete *et al*., 2014), *Sternbergia candida* (Parmaksiz and Khawar, 2006) and *Cicer milkvetch* (Uranbey *et al*., 2003). In support of the present result, Khalid *et al*. (1989) reported higher number of shoots at lower concentration of NAA. As NAA concentration increased, a decrease in regeneration capability was observed. This might be due to the fact that NAA is known for its root formation ability and usually doesn't respond well to shoot proliferation (Hongwei, 1994). However, in many legume species, high concentration of cytokinin combined with low concentration of auxin reportedly induced better shoots than a medium supplemented with cytokinin alone (Susan *et al*., 1998).

Rooting and acclimatization

Auxins are mainly used in root induction and their effect varies with type and concentrations used in different plant species (Swamy *et al*., 2002). It has been repeatedly confirmed that auxin is required for initiation of adventitious roots on stems and the development of the first initial root cells is dependent upon either the exogenous or the endogenous auxins (Pignatti and Crobeddu, 2005; Radi *et al*., 2013).

In the present study, the analysis of variance revealed that root number and root length varied significantly on half strength MS medium supplemented with different concentrations of NAA, IAA and IBA. The best rooting potential in terms of mean number of roots and root length was obtained on medium containing NAA when compared to IAA or IBA. Ye *et al*. (2002) reported that NAA is better than IBA for rooting of lentil. The highest number of roots (18.36±1.28) with the highest mean root length (3.43 ±0.13 cm) was achieved on half-strength MS medium containing 0.1 mg/l NAA. Sroga (1987) also reported similar result for *L. angustifolius*. Upadhyaya *et al*. (1992) recommended low concentration of NAA for maximum root number.

Acclimatization of *in vitro* rooted plantlets was successful, where 90% plants survived and established as healthy plants. No aberrant plants were observed. This result was better than previous report by Upadhyaya *et al*.

(1992) where the survival rate of *L. texensis* plants was only 50%.

CONCLUSION

Sterilization of *L. albus* seeds with 30% (1.5% active chlorine) sodium hypochlorite for 20 min exposure time and 20% (1% active chlorine) for 25 min exposure time is very effective to obtain contamination-free seeds. Medium containing 1.0 mg/l BAP was found to be the most effective for shoot initiation whereas medium containing 2.0 mg/l BAP was the best for shoot multiplication. For rooting, 0.01 mg/l NAA was found to be the best concentration. The protocol developed in this study is an important starting point for further genetic improvement of this species using biotechnological approaches because *in vitro* propagation protocol is a prerequisite for further applications of plant tissue culture such as *in vitro* regeneration, genetic transformation and genome editing.

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