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ORIGINAL PAPER

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BREAKING SEED DORMANCY AND MICROPROPAGATION OF PERENNIAL VULNERARIA MILKVETCH (*Astragalus vulnerariae* DC.)

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ABSTRACT

In this study, an efficient system to break seed dormancy and plant regeneration was established for perennial vulneraria milkvetch (*Astragalus vulnerariae* DC.). The seed coat dormancy could be easily released using 40% sulphuric acid treatment for 15 minutes, which made the seed coat permeable without damaging zygotic embryos. The tissue culture studies reported regeneration on 5 explants, which had variable effects on shoot regeneration, using MS medium containing variable concentrations of Kin–NAA and BAP–NAA. Maximum shoot regeneration of 86.67% with 4.47 shoots per explant was noted on MS medium containing 0.5 mg·l^{-1} Kin – 0.5 mg·l^{-1} NAA on hypocotyl explants. No shoot regeneration was noted on cotyledon leaf and shoot nodes using MS medium containing any concentration of BAP–NAA. Maximum shoot regeneration of 50% with 3 shoots per explant was also observed on 1 mg·l^{-1} BAP – 0.5 mg·l^{-1} NAA on epicotyl explants. MS medium containing Kin–NAA induced hypocotyl explant shoots had rooting percentage of 36.67% on 0.5 mg·l^{-1} IBA, whereas, MS medium containing BAP–NAA induced hypocotyl shoots had rooting percentage of 46.67%. The *in vitro* cultured plants had an acclimatization rate of 78%. Aesthetically attractive, economical, easy to maintain and water efficient vulneraria milkvetch is currently not available in landscaping. The system of plant regeneration and adaptation suggested by this paper may help to dissipate it to a broad range of water scarce environments as a sustainable and inexpensive choice.

Key words: *Astragalus vulnerariae* DC., vulneraria milkvetch, seed dormancy, micropropagation, *in vitro*

INTRODUCTION

Astragalus is an economically important genus of the family Leguminosae that is represented by 380 species in the flora of Turkey [Chamberlain and Matthews 1970, Tubives 2016]. The plants belonging to the genus have great potential for arid urban landscaping and for the production of high quality forages [Cho et al. 2000], which could be used for the establishment of rangelands and artificial meadows. They could also be used in gum production and as medicinal plants [Rios and Waterman 1998], since they are rich in polysaccharides, saponins, and isoflavonoids [Bedir et al 2000].

Perennial endemic legume *Astragalus vulnerariae* DC (vulneraria milkvetch) is an important plant species belonging to this genus, which has attractive yellow flowers. It is endemic to the arid lands of Marmara and Central Anatolian regions of Turkey [Chamberlain and Matthews 1970, Tubives 2016].

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Vulneraria milkvetch has a very high potential for use in arid urban landscaping as they are highly resistant to drought and grow on lands that receive less than 400 mm of annual rainfall. Use of this plant in urban arid landscaping can help in terms of water and soil conservation of Central Anatolia. It may also contribute to the creation of harmony with local environmental conditions needing minimum maintenance and planning that could lead to preferred economic approach/approaches [Akdoğan 1972].

Vulneraria milkvetch seeds have hard-seed coats, which make seed germination very difficult. Germinated seeds face difficulties in competing with weeds and harsh external environmental conditions during the earlier stages of growth (Authors' observations). Therefore, new strategies, including plant biotechnology, are needed to improve the seed germination and growth of these important plants, so that they could be used for making the environment greener.

Plant biotechnology can be used as an alternative to solve this problem. Previous studies report *in vitro* micro-propagation of North American species *A. columbianus*, *A. amblytropis*, *A. aquilonius* and *A. mulfordiae* [Edson et al. 1998]; *A. adsurgens* [Luo and Jia 1998a, Luo et al. 1999], *A. cicer* [Uranbey et al. 2003, Basalma et al. 2008] using different explants. Plant regeneration of *A. adsurgens* through protoplast culture has also been reported by Luo and Jia [1998b].

This study aimed to break seed dormancy and establish an *in vitro* shoot regeneration system for vulneraria milkvetch, which has not been reported earlier. It is expected that this will offer a potential for rapid multiplication of the plant, which carries importance as an alternative for dry lands urban landscaping in areas receiving limited rainfall.

MATERIALS AND METHODS

Plant material. The seeds of *A. vulnerariae* were collected from Beynam and its environs (Ankara, Turkey) within the scope of a research project supported by TUBITAK (2209) on "Seed Collection of Some Species of *Astragalus* L. to be Used as Propagation Materials around Ankara" during 2012.

Identification of seed viability using tetrazolium test. The seed vitality test was performed after modifying the modified protocol of Sumlu et al. [2010]. The seeds were opened at opposite end to the embryonic axis using a stainless steel scalpel blade No. 10 giving a superficial cut and immersed in 1% tetrazolium solution placed in falcon tubes for 24 hours in the dark at room temperature. Thereafter, the testas were gently removed without damaging the seeds and both cotyledons were gently separated longitudinally so that embryo could be observed on one edge of two cotyledons. Subsequently, the embryo was also gently removed from the cotyledons. Formazan red tones were noted on cotyledons and embryos. The presence of red areas showed vitality and white areas showed non viability. The seeds were counted dead if the embryos did not stain red.

Seed dormancy break and surface sterilization. Since the seeds were difficult to germinate due to their hard coats, they were treated with 10, 20, 30 and 40% sulphuric acid for 15 minutes to optimize the best concentration of the acid for breaking seed dormancy and achieving higher seed germination rate. Thereafter, the seeds were rinsed three times with autoclaved distilled water before subjecting them to surface sterilisation with 100% commercial bleach (Ace, Turkey, containing 5% NaOCl) and later with sterilised water for 5 × 3 min. All sterilised seeds were cultured on 35 ml of MS [Murashige and Skoog 1962] medium supplemented with 30g·l^{-1} sugar, solidified with 0.65% agar (Duchefa) and incubated in the growth room for germination at 24°C, at 16 h light (35 μmol photons m^{-2} s^{-1} provided by Philips daylight lamps (TLD 36 W/54, Hungary) 8 h dark photoperiod to evaluate their efficiency in germination.

The 10-day-old seedlings were used for obtaining hypocotyl, epicotyl, cotyledon leaf, stem node and leaf explants for use in tissue culture studies.

These explants were cultured on MS medium containing 0.5, 1, 1.5 and 2 mg·l^{-1} Kin – 0.5 mg·l^{-1} NAA; 0.5, 1, 1.5 and 2 mg·l^{-1} BAP – 0.5 mg·l^{-1} NAA supplemented with 30 g·l^{-1} sucrose and solidified with 0.65% agar (Duchefa) in Magenta GA7 vessels and incubated in the growth room for regen-

eration at 24°C, at 16 h light (35 µmol photons m^{-2} s^{-1}) and 8 h dark photoperiod.

The 3–4 cm long individual micro shoots induced on 5 types of explants were aseptically cut and rooted under *in vitro* conditions on MS medium containing 0.5 mg·l^{-1} IBA on agar solidified MS medium supplemented with 2.5% sucrose and 3 g·l^{-1} activated charcoal for 42 days in Magenta GA7 Vessels under 16 h light photoperiod. These were evaluated for percentage of rooting, mean number of roots per explant and root length.

The pH of all culture media was adjusted to 5.6–5.8 using 1 N KOH or 1 N HCl before adding agar. The cultures were autoclaved for 20 min at 121°C and 104 kPa. All rooted plants were planted in peat moss after removing agar from the roots under tap water. The rooted plants were first hardened under *in vitro* conditions by transferring the plants to small PET glasses containing 1/8 × liquid MS medium that lacked sucrose for 60 h. Afterwards, the rooted plantlets were transferred to plastic pots and were acclimatized to the external environmental conditions at 24 ±2°C in the glass house under 16 h light photoperiod and average 60% relative humidity.

Statistical analysis. The data on shoot regeneration, rooting and acclimatization were evaluated after 60 days of culture. Each plant growth regulator treatment had 6 replicates containing 60 explants. One-way ANOVA and Duncans multiple range test IBM SPSS 19.0 for windows were used to analyse the statistical data.

RESULTS

Seed dormancy

The tetrazolium test carried out with 400 seeds showed the seeds vitality of 89.90% (data not shown). No seed germination was noted on untreated seeds on agar solidified MS medium (control). Lower concentrations of sulphuric acid made seed coat less permeable to external water and oxygen that prolonged the time for seed germination. Minimum seed germination percentage (65.40%) was noted on seeds treated with 10% sulphuric acid after 25 days. Each increase in the concentration of sulphuric acid was followed by a corresponding increase in seed coat permeability and a reduction in germination period in days. Seed treatment with 40% sulphuric acid was optimum and sufficient to release seed dormancy resulting in 88.95% seeds germination (approximately equivalent to tetrazolium test results) in 7 days that affirmed seed vitality (tab. 1).

Table 1. Breaking seed dormancy of vulneraria milkvetch seeds by sulfuric acid treatment with seed coat breaking

Sulphuric acid concentration in percentage (%)	Germination percentage (%)	Number of days to germinate
10	65.40c	25
20	70.32b	21
30	87.50a	16
40	88.95a	7
Control	0.00d	0

Values shown by different letters in a single column are significantly different in accordance with Duncan's multiple range test at 0.05 level of significance

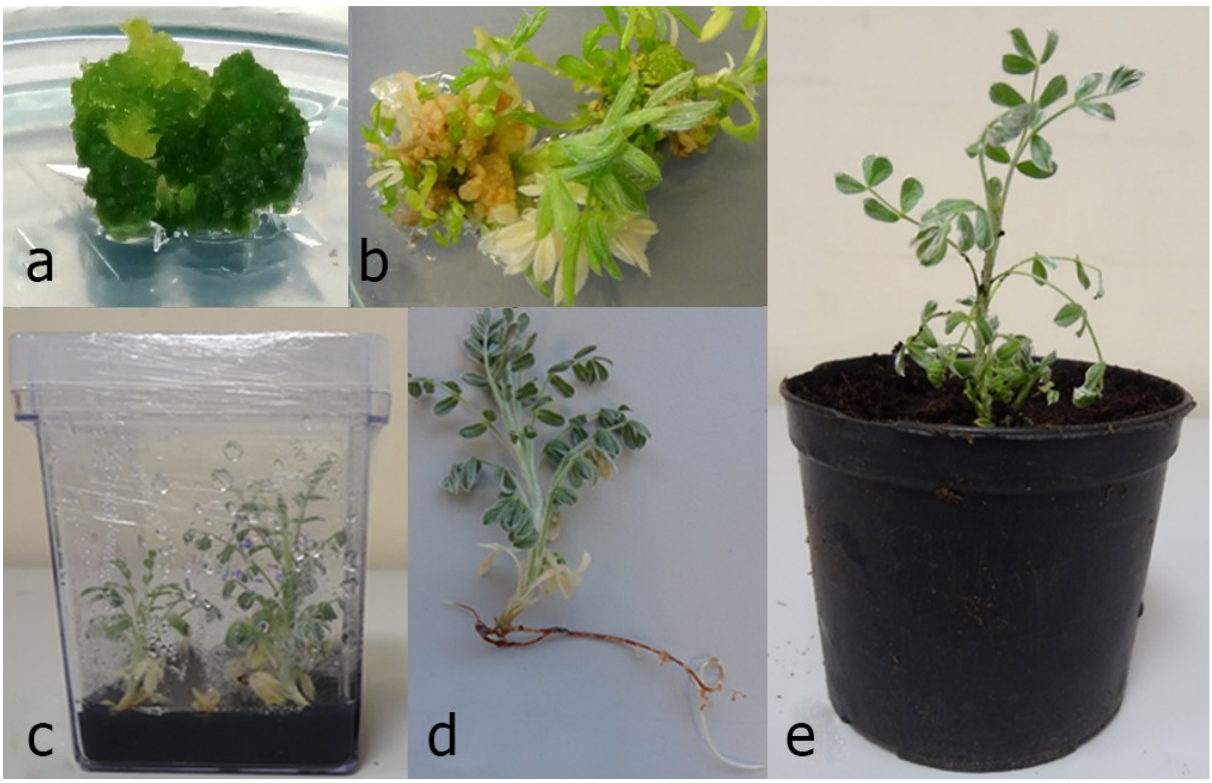
Effects of different concentrations of Kin–NAA on shoot regeneration

The results of the study showed that all combinations of Kin–NAA were suitable for *in vitro* shoot regeneration from all types of explants tested (hypocotyl, epicotyl, cotyledon leaves, shoot nodes, and leaf) irrespective of their origin.

Callus regeneration was noted on each type of explant after 8–20 days of culture (data not shown). The earliest callus regeneration was noted on hypocotyl explants; which induced callus based shoot initials after about 24–25 days (phot. 1a), where shoot regeneration percentage, number of shoots per explant and shoot length were induced with significant differences ($p < 0.05$) among themselves under the influence of tested concentrations of Kin–NAA.

Hypocotyl, epicotyl and cotyledon leaf explant showed shoot regeneration on all concentrations of Kin–NAA that ranged between 26.67–86.67%, 13.33–53.33% and 20.00–53.33% respectively. Shoot regeneration decreased consistently with increase in the concentration of Kinetin from hypocotyl and epicotyl explants. Maximum and minimum shoot regeneration percentage on both hypocotyl and epicotyl explants was noted on 0.5 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA (phot. 1b) and 2 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA respectively. Shoot regeneration percentage was inconsistent on cotyledon leaf explants (tab. 2).

Shoot node explants induced shoot regeneration on two Kin–NAA concentrations only. No shoot induction was noted on 0.5 and 1 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA.



Phot. 1. Micropropagation of vulneraria milkvetch: (a) callus regeneration on hypocotyl explants with shoot initials after about 24–25 days; (b) maximum shoot regeneration percentage on hypocotyl explant as noted on MS medium containing 0.5 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA; (c, d) rooting on 0.5 $mg \cdot l^{-1}$ IBA after 6 weeks of culture; (e) acclimatized vulneraria milkvetch plant in glass house

Table 2. Effects of various concentrations of Kin–NAA on shoot regeneration from five different types of vulneraria milkvetch explants

	Treatments		Explants				
	Kin ($mg \cdot l^{-1}$)	NAA ($mg \cdot l^{-1}$)	Hypocotyl	Epicotyl	Cotyledon leaf	Shoot nodes	Leaf
Shoot regeneration percentage (%)	0.50	0.50	86.67a	53.33a	33.33b	0.00c	0.00b
	1.00	0.50	73.33b	40.00b	53.33a	0.00c	0.00b
	1.50	0.50	66.67c	33.33c	20.00d	20.00b	26.67a
	2.00	0.50	26.67d	13.33d	33.33c	33.33a	0.00b
Mean number of shoots per explant	0.50	0.50	4.47a	3.0a	1.25c	0.00c	0.00b
	1.00	0.50	4.00b	2.33b	2.67a	0.00c	0.00b
	1.50	0.50	3.20c	1.37c	1.33c	1.54b	1.33a
	2.00	0.50	1.26d	1.52d	1.67b	1.67a	0.00b
Shoot length (cm)	0.50	0.50	5.04b	4.01a	2.33c	0.00c	0.00b
	1.00	0.50	5.47a	4.06a	2.45c	0.00c	0.00b
	1.50	0.50	4.20c	3.22b	2.67b	1.33b	2.75a
	2.00	0.50	1.26d	1.93c	4.40a	1.39a	0.00b

Values shown by different letters in a single column are significantly different in accordance with Duncan's multiple range test at 0.05 level of significance

Whereas, leaf explant induced shoot regeneration of 26.67% on MS medium containing 1.5 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA only (tab. 2).

Number of shoots per explant ranged 1.26–4.47, 1.37–3.0 and 1.25–2.67 on hypocotyl, epicotyl and cotyledon leaf explants respectively (tab. 2).

Maximum number of hypocotyl and epicotyl induced shoots per explant were noted on 0.5 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA. Whereas, maximum number of shoots per explant on cotyledon leaf explant was noted on 1 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA. Shoot node explant induced maximum number of 1.67 shoots per explant on 2 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA while leaf explant induced mean number of 1.33 shoots per explant on 1.5 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA (tab. 2).

Shoot length on hypocotyl, epicotyl and cotyledon leaf explants ranged 1.26–5.47, 1.93–4.06 and 2.33–4.40 cm respectively. Increased concentration of Kin reduced the length of shoots regenerated from hypocotyl and epicotyl explants; whereas, each increase in the concentration of Kin induced increased length of shoots on cotyledon leaf explants. Cotyledon leaf induced longest shoots of 1.39 cm were noted on MS medium containing 2 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA. Whereas, leaf explant induced average shoot length of 2.75 cm (tab. 2).

Rooting. Hypocotyl, epicotyl, cotyledon leaf, shoot node and leaf explants showed maximum shoot regeneration on 0.5 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA, 0.5 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA, 1 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA, 2 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA and 1.50 $mg \cdot l^{-1}$ Kin – 0.5 $mg \cdot l^{-1}$ NAA respectively. Therefore, these shoots (regenerated on best induction medium) were rooted on 0.5 $mg \cdot l^{-1}$ IBA.

Table 3. Rooting of vulneraria milkvetch shoots from different explants regenerated on Kin–NAA medium on 0.5 mg·l^{-1} IBA

Explant	Rooting percentage (%)	Mean number of roots per explant	Root length (cm)
Hypocotyl	36.67a	2.33a	3.55a
Epicotyl	30.82b	1.28b	1.83b
Cotyledon leaf	20.08c	1.00c	1.69c
Shoot nodes	18.91cd	1.84d	1.61c
Leaf	14.96d	1.47e	1.54c

Values shown by different letters in a single column are significantly different in accordance with Duncan's multiple range test at 0.05 level of significance

All shoots induced and grew well developed roots within 6 weeks (phot. 1c, d).

Rooting percentage ranged between 14.96–36.67%. The best rooting was noted on hypocotyl induced shoots with 2.33 roots per explant with average root length of 3.55 cm. The minimum rooting was noted on leaf induced shoots with 1.47 roots per explant and average root length of 1.54 cm (tab. 3).

Hardening and acclimatization. The *in vitro* regenerated plants placed in the glass house in plastic pots had a cumulative survival rate of 78% (phot. 1e). All of the established plants showed high uniformity and similarity without any detectable variation on their phenotypes, when compared to non-tissue cultured plants.

Effects of different concentrations of BAP–NAA on shoot regeneration

All explants showed regeneration variably. A comparison of the results showed that combinations of BAP–NAA containing MS medium were less inductive compared to Kin–NAA containing MS medium.

Hypocotyl explant induced shoot regeneration of 30.67% on single medium containing 1.5 mg·l^{-1} BAP – 0.5 mg·l^{-1} NAA with mean number of 1.67 shoots/explant and a shoot length of 1.60 cm (tab. 4).

Epicotyl explant induced shoot regeneration of 46.67–50% on two regeneration media containing 0.5 mg·l^{-1} BAP – 0.5 mg·l^{-1} NAA and 1 mg·l^{-1} BAP – 0.5 mg·l^{-1} NAA. It induced maximum number of 3 shoots with maximum shoot length of 4.93 cm (tab. 4).

No shoot regeneration was noted on cotyledon leaf and shoot node explants.

Leaf explant also induced shoot regeneration of 26.67–46.67% on three regeneration media. It induced maximum shoot regeneration of 46.67%, with 3.67 shoots per explant on MS medium containing 0.5, 1 mg·l^{-1} BAP – 0.5 mg·l^{-1} NAA. The explant induced maximum shoot length of 4.67 cm on MS medium containing 1.5 mg·l^{-1} BAP – 0.5 mg·l^{-1} NAA (tab. 4).

Rooting. No shoot induction was noted on cotyledon leaf and shoot node explants. Hypocotyl, epicotyl, and leaf explants showed maximum shoot regeneration on 1.5 mg·l^{-1} BAP – 0.5 mg·l^{-1} NAA, 1 mg·l^{-1} BAP – 0.5 mg·l^{-1} NAA, 0.5 mg·l^{-1} BAP – 0.5 mg·l^{-1} NAA respectively. Therefore, these shoots (regenerated on best induction medium) were rooted on 0.5 mg·l^{-1} IBA. All shoots induced well developed growing roots within 5–6 weeks.

Rooting percentage ranged between 32.63–46.67%. Again the best rooting was noted on hypocotyl induced shoots with 2.49 roots per explant and

Table 4. Effects of various concentrations of BAP–NAA on shoot regeneration from five different types of vulneraria milkvetch explants

	Treatments		Explants				
	BAP ($mg \cdot l^{-1}$)	NAA ($mg \cdot l^{-1}$)	Hypocotyl	Epicotyl	Cotyledon leaf	Shoot nodes	Leaf
Shoot regeneration percentage (%)	0.50	0.50	0.00	46.67	0.00	0.00	46.67
	1.00	0.50	0.00	50.00	0.00	0.00	30.67
	1.50	0.50	30.67	0.00	0.00	0.00	26.67
	2.00	0.50	0.00	0.00	0.00	0.00	0.00
Number of shoots per explant	0.50	0.50	0.00	2.67	0.00	0.00	3.67
	1.00	0.50	0.00	3.00	0.00	0.00	1.90
	1.50	0.50	1.67	0.00	0.00	0.00	1.67
	2.00	0.50	0.00	0.00	0.00	0.00	0.00
Mean shoot length (cm)	0.50	0.50	0.00	4.93	0.00	0.00	2.83
	1.00	0.50	0.00	2.84	0.00	0.00	2.54
	1.50	0.50	1.60	0.00	0.00	0.00	4.67
	2.00	0.50	0.00	0.00	0.00	0.00	0.00

Table 5. Rooting of vulneraria milkvetch shoots from different explants regenerated on BAP–NAA medium on 0.5 $mg \cdot l^{-1}$ IBA

Explant	Rooting percentage (%)	Mean number of roots per explant	Root length (cm)
Hypocotyl	46.67	2.49	5.09
Epicotyl	40.31	1.16	4.64
Leaf	32.63	1.69	4.19

an average root length of 5.09 cm. The minimum rooting was noted on leaf induced shoots with 1.69 roots per explant with average root length of 4.19 cm (tab. 5).

Hardening and acclimatization. The *in vitro* BAP–NAA recovered plants showed a cumulative survival rate of 55%. These plants indicated high consistency and similitude with no distinguishable minor departure from their phenotypes when checked against seed grown plants.

DISCUSSION

Seed coat mechanical or physical dormancy that hinder water and oxygen permeability through hilum to embryos is a major problem in legumonous plants including Astragalus species [Baskin and Baskin 1998, Baskin 2003, Pipinis et al. 2011]. The seed germination period decreased consistently with increased seed coat permeability after weakening of seed coats with each increase in concentration of

sulphuric acid. The effects of sulfuric acid scarification treatments depended upon the soaking concentration. While 10–30% sulfuric acid scarification poorly affected impermeability through hilum and seed coat [Riggio-Bevilacqua et al. 1985]; the rate of seed germination was almost equivalent to the tetrazolium test based seed vitality after 40% sulphuric acid scarification. Thus, this concentration increased maximum permeability of oxygen and water to embryos and activation of metabolism (after contact with embryos) resulting in easy cracking of seed coat and hilum followed by emergence of radicle, plumule and accelerated growth of seedlings in 7 days, in agreement with Riggio-Bevilacqua et al. [1985], Wagner and Spira [1994], Pipinis et al. [2011] and Azizi and Changaie [2013].

Moreover, the germination percentage achieved at 40% scarification was very close to the seed viability test percentage; therefore, it could be safely concluded that the sulphuric acid concentration did not cause any injury to the seed embryos. The method of seed germination developed in this study could be used for healthy seed multiplication in nurseries independent of external biotic or abiotic stresses that hinder seed germination under natural conditions.

The major problem to regeneration from any explant could be the right choice of the explants, which could be counted as their normal and primary reaction. This study evaluated the responses of 5 different types of explants on different concentrations of Kin–NAA and BAP–NAA. Although several studies have reported *in vitro* regeneration in leguminosae using different explants; there is no study that reports regeneration from hypocotyl, epicotyl, cotyledon leaf, stem node, and leaf explants in vulneraria milkvetch. Depending on the level of totipotency, the growth regulator concentrations affected the cells of explants leading to their division growth and organogenesis variably.

Although the response of tissue culture on KIN–NAA and BAP–NAA regeneration media varied, it was evident that regeneration depended on both explant and growth regulator. The highest shoot regeneration on Kin–NAA medium was noted from hypocotyl explants; whereas, the highest shoot regeneration on BAP–NAA medium was noted from epicotyl explant. The results are in partial agreement with Başalma et al. [2008], who suggests the use of hypocotyl and cotyledon explants for efficient and reproducible plant regeneration from cicer milkvetch. Başalma et al. [2008] further suggested that hypocotyl explants of cicer milkvetch were more responsive compared to cotyledon explants on MS medium containing TDZ. The researchers also observed regeneration of calli before induction of shoots. They found that cotyledon explant was recalcitrant compared to hypocotyl explant. Shoot regeneration behavior in this study also showed variation depending on the type of plant – growth regulator combinations in agreement with Uranbey et al. [2003], who reported regeneration from cicer milkvetch. Comparing the results of this study with the studies by Gu [1987], Fakhrai and Evans [1989] and Uranbey et al. [2003] clearly indicate that the type of explant and shoot regeneration medium are very important for optimum regeneration.

Both Indole and Naphthalene acetic acid are popularly used for rooting of *in vitro* regenerated shoots [Özel et al. 2008]. No problem was noted on rooting of *in vitro* regenerated shoots from hypocotyl and epicotyl explant using 0.5 $mg \cdot l^{-1}$ IBA. This result is not in agreement with Uranbey et al. [2003], who noted 40% rooting of *in vitro* regenerated cicer milkvetch on 1/2 × MS medium containing 5.4 µM NAA. Use of this system of regeneration could help in easy multiplication of the plants for arid landscaping nurseries.

The most important step in *in vitro* cultured plants is their acclimatization to the external conditions. There are significant differences in the environments under *in vitro* conditions compared to the external environment in greenhouse or glass house in terms of relative humidity, temperature, quantity and quality of lighting availability of nutrient elements, gaseous composition and the medium substrate etc. Slow plant growth and significant plant losses occur [Seelye et al. 2003, Mokhtarzadeh et al. 2013], if proper care is not taken during initial periods of plant transfer (to external environment). Contrarily, the results of this study showed that the *in vitro* regener-

ated plants had no problem in hardening and acclimatization on peat moss in the glass house.

CONCLUSION

The establishment of a highly efficient seed dormancy break and plant regeneration system of *Astragalus vulnerariae* DC. is reported in this study for the first time. This protocol enabled researchers to obtain plants from both seeds and 5 different types of explants under *in vitro* conditions within 136 days. A comparison of Kin–NAA and BAP–NAA containing media shows that Kin–NAA could be favoured for high rate of shoot multiplication from hypocotyl and BAP–NAA on epicotyl explant. It is expected that present protocol could help in easy and rapid multiplication of *Astragalus vulnerariae* DC. for ornamental dry land and landscaping nurseries in an economic way.

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