Response of *Prosopis koelziana* Burkart to *in vitro* culture and salt stress

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Abstract

Effect of explants source, plant growth regulator and NaCl on organogenesis and proline accumulation in *prosopis Kolziana*, salt tolerant species, were studied. Meristem, young leaf, and cotyledon separated from *in vitro* seedling. Then explants were cultured on MS medium supplement with different plant growth regulators. Multiple shoot regeneration was observed in meristem explants. In contrast leaf callus did not produce any shoot and root. The best treatment for shoot regeneration was MS Medium containing 1 mgl^{-1} 6-benzylaminopurine and 0.1 mgl^{-1} naphtalenacetic acid. Auxins promoted callus and root regeneration. In contrast cytokinin hormones induced shoot formation. Effect of different concentrations of NaCl (2, 4, 6, 8, 10 gl^{-1}) on organogenesis and proline accumulation were investigated. The result showed salt stress tends to decrease of organogenesis. But it had less effect on callus induction. Although, high content of NaCl inhibited both callusing and shoot regeneration. Proline content was significantly increased by increasing the NaCl. Increasing the NaCl concentration from 8 mgl^{-1} to 12 mgl^{-1} did not enhance proline content. It is suggested proline had an important role on salt resistance in this species.

Key words: Explant source, PGR, Organogenesis, Callusing, Proline accumulation, *Prosopis Kolziana*

Abbreviation: BAP: 6-benzylaminopurine, IBA: indol-3-butyric acid, Kin**:** kinetin**,** 2iP**:** 2-Amino purine NAA: naphtalenacetic acid, IAA: indol acetic acid, MS: Murashige and Skoog medium, min: minute, PGR: plant growth regulator

Introduction

Salinity is a major factor limiting the crop productivity in the arid and semi-arid areas of the world (Ashraf, 1994). Prosopis genus is grown in semi-arid region of the Americas, Africa and Asia. These plants are seen to survive and grow with salinity level equal to that of sea water (Felker *et al*. 1981b). They are as a source of firewood and charcoal. Pods of prosopis species are a valuable source of carbohydrate and protein. *Prosopis Kolziana*, a salt tolerant species, is an intermediate species between *Prosopis cineraria* and *Prosopis farcta.* It is grown in Iran and Saudi Arabica.

Selection of favorable somaclonal variant strains from callus culture is appropriate tools for production of stress resistance plant (Larkin and Scowcroft 1981, Dix 1993, Ashraf 1994). *In vitro* propagation of *prosopis* species was conducted by (Tabone *et al*. 1986, Jordan *et al*. 1987, Batchelor *et al*. 1989, Yao *et al*. 1989, Walton 1990, Arceand Balboa 1991, Felker 1991 and Hammod

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1991. *In vitro* selection of salt tolerant cell line and proline accumulation under salt stress were reported in several species such as tomato species (Cano *et al*. 1996 and 1998), *Populus euphratia* (Watanabe *et al*. 2000), *Arachis hypogaea* L. (Jain *et al*. 2001), *Actinidia deliciosa* (Sotiropoulos and Dimassi, 2005), *Eucalyptus camapdupensis* (Wood ward and Bennett, 2005), *Solanum tuberosum* L. (Zhang *et al*. 2006), Apple rootstock (Malassiotis *et al*. 2006) and *Phaseolus vulgaris* L. (Jimenez-Bremont *et al*. 2006).

The objectives of this study were to determine effects of explants source and plant growth regulator on callus induction and subsequent organogenesis and also evaluate *in vitro* salt tolerance in *Prosopis koelziana* Burkart.

Material and Methods

Plant material

Seeds of *Prosopis Koelzina* were collected from Shahdad Bam and maintained in 4ºC. Then, they were scarified with concentrated sulfuric acid for 5, 10, 15, 20 min and washed 5 times with sterile distilled water. In order to produce sterile seedling, seeds cultured on MS (Murashige and Skoog, 1962), B5 and SH media without plant growth regulator. Seeds were cultured on honey jar containing 100 ml of culture medium. After 30–40 days seedling with 5 or 6 leaves were selected.

Callus induction and organogenesis

Explants included meristem, hypocotyls and young leaves separated from seedling. After surface sterilization, explants cultured on MS medium. Effects of different auxins (2,4-D, NAA and IAA) and cytokinins (BAP, Kin and 2iP) with (0.05, 0.1, 0.5, 1, 2 mgl^{-1}) concentration on callus induction and organogenesis evaluated. In next step the interactive effect of BAP and NAA were studied too.

In vitro salt treatment

Explants via meristems, hypocotyls and young leaves, were transferred to the best shoot regeneration medium (1 mgl^{-1}BAP and 0.1 mgl^{-1} NAA) containing 0, 2, 4, 6, 8, 10 and 12 gl^{-1} NaCl. After 40 days callusing and shoot regeneration in different treatment were compared.

Proline assay

Proline was extracted from 0.5 g fresh plant material as described by (Bates, 1973). The amount of proline was measured by spectrophotometer with 520 nm wave length. Then proline content was determined according to calibration curve prepared with a seris of standard proline solution.

Culture condition and observation

Cultures maintained at 25±2ºC with 16h photoperiod provided by Double cool fluorescent tube with 1000 lux intensity**.** Each treatment consisted of 30 sample and after 40 days quality of callus, shoots and roots were evaluated. For each trait gave a number according to tables

Result

Callus induction and organogenesis

Maximum germination rate (78.88) was achieved by 10 minutes soak in 98% sulphuric acid (figure 1). Among different explants, meristems were the most appropriate samples for shoot and root regeneration. In hypocotyls explants organogenesis was done too. On medium containing auxin, root and shoot induction occurred in hypocotyls explants. However cytokinins inhibited root regeneration and decreased shoot formation in this explant. In leaf explants organogenesis did not promote (Table1 and 2). 2, 4-D induced callus formation in all explants. Maximum organogenesis was obtained in meristem and hypocotyle. In medium containing NAA, meristem produced more calli. Among different auxin, NAA promoted more root regeneration. High amount of IAA induced callus and stimulated organogenesis. The result showed all auxin promoted cell proliferation and callus formation (Table 1).

BAP stimulated cell proliferation, callusing and organogenesis. Chlorophyll content increased in samples by adding BAP. 2iP promoted callusing and organogenesis at high level in comparison to BAP. Kin induced callus in all explants. This growth regulator was effective on shoot regeneration. But it prevented root regeneration (Table 2). In comparison among cytokinin, Kin had weak effect on organogenesis and callus volume was decreased. Result showed all cytokinins produced more shoot than auxins. In

contrast they had an inhibitory effect on root regeneration. In medium containing BAP and NAA, callus induced in all treatment. Explants produced more shoots when BAP to NAA ratio was 10. The maximum shoot regeneration was obtained in medium with 1 mgl^{-1} BAP and 0.1 mgl^{-1} NAA (figure 2a). Root regeneration was increased by enhancement of NAA concentration (Table 3).

Effect of salt Stress

NaCl influenced on callus induction and organogenesis. Salt stress inhibited shoot regeneration at lower content. However callus formation was achieved at higher concentration. Produced shoots under salt stress showed chlorosis and wilting symptoms in meristem explant. Organogenesis was decreased in hypocotyle explant at lower NaCl concentration (figure 2b). High content of NaCl led to death of explant in hypocotyle (Table 4). Proline content significantly increased by increasing of NaCl concentration up to $6gl^{-1}$. However there was no significant difference among 8, 10 and $12gl^{-1}$ NaCl concentration (figure 3).

Discussion

Callus induction and organogenesis:

Seed germination was achieved in all media. This phenomenon showed ability of prosopis to growth and development in wide range of nutritional element. Scarification of seeds improved germination rate. It led to increasing of seed coat permeability and encouraged germination. The best time for H_2SO_4 treatment was 10 minutes. Maximum germination rate of over 95% was achieved with *prosopis juliflora* seeds by a 15 or 30 minutes soak in 97% H_2SO_4 (Pasiecznik *et a*l., 1998). The most suitable explants for shoot and root regeneration were meristem. Maximum callusing was in meristem too. Adding auxin to medium enhanced cell proliferation and callusing. 2,4-D influenced on callus induction strongly. NAA promoted either callusing and root regeneration. IAA in comparison with two other auxin had weak effect on callus formation and organogenesis. It was effectiveness in 5-fold concentration of NAA or 2, 4-D. Low ability of IAA is due to IAA destruction. IAA oxidase and light are responsible for IAA degradation (Dale, 1975). Cell proliferation, callus formation, new shoot production as well as increasing of chlorophyll content were observed by adding cytokinin. Root regrneration was achieved rarly Iin medium containing cytokinin. Among cytokinin, BAP was more effective in relation to organogenesis. Increasing of 2iP up to 4-fold of BAP improved shoot regeneration. These results are in agreement with Koda, 1980. Interactive effect of BAP and NAA increased callusing and organogenesis. The highest shoot regeneration (7 shoots per explant) was observed in medium with 1 mgL^{-1} BAP and 0.1 mgL^{-1} NAA. This result is in agreement with Nandwani (1992), Singh and Rothore (1993) in *Prosopis tamarugo* and *P. cineraria* respectively.

Effects of salt stress

In all explants Organogenesis as well as callus induction were decreased under salt stress. At high content of NaCl shoot regeneration and callus induction were inhibited. Serrato valenti *et al*., 1992 showed at 600 mM NaCl concentration, germination of *P. cineraria* L. was greatly hindered. They also considered salinity induced structural change in the roots, hypocotyls, epicotyls and leaflet. Plants had cholorosis and wilted appearance when they exposed to salt stress. Photosynthesis rate of leaf is limited during salt stress. It seemed NaCl inhibited chlorophyll synthesis. A significant accumulation of Proline was observed by increasing of NaCl concentration. Increasing of proline content under salt strees were considered by Sane et al. 2005 in Date palm and Watanabe *et al*. 2000 in *Populus Euphratia*. Jain et al., 2001 showed accumulation of proline which was probably associated with osmotic adjustment and the protection of membrane integrity. Salinity stress increased superoxide dismutase and peroxidase activity in *Lycopersicon Pennellii* which are inhibited cell membrane damage (KoKa and Turkan, 2006). Adition of Proline to the culture medium alleviated the salt stress and reduced peroxidative damage of the lipid membrane in Groundnut (Jain, 2001). It should be suggested proline had an important role on salt resistance in this species.

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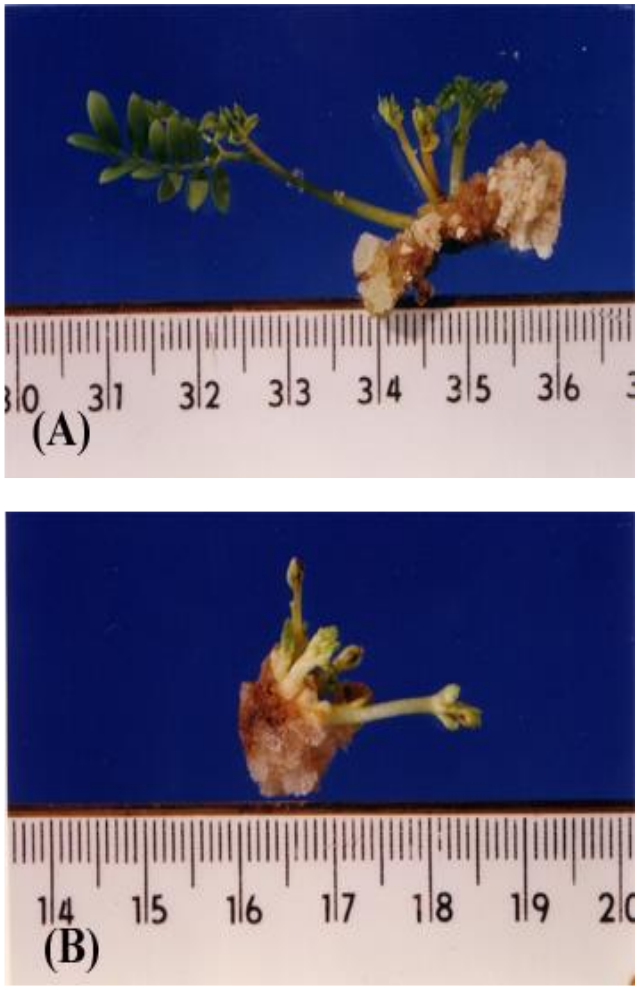

Figure 2: (A) callus formation and organogenesis in meristem explant on medium containing 1 mgl^{-1} BAP and 0.1mgl^{-1} NAA. (B) Effect of salt stress on callus induction and shoot regeneration.

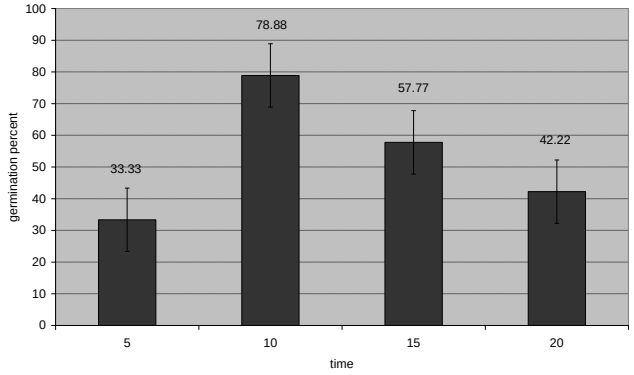

Figure 1: Seed germination under different H_2SO_4 time treatment (minute)

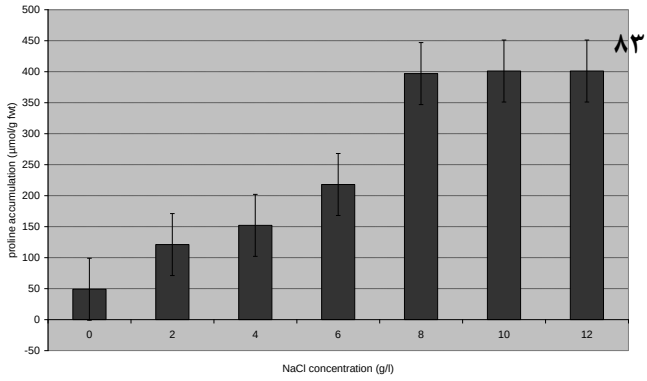

Figure 3: comparison of proline content in different NaCl concentration

Table1: The Effect of explants Source and different auxin on callus formation and organogenesis *in Prosopis koelziana* Burkart

PGR	Meristem			hypocotyl			leaves		
	callus	shoot	root	callus	shoot	root	callus	shoot	root
2.4-D									
0.05	2	1	1	2	2	0	2	0	0
0.1	4	2	3	3	3	1	3	0	0
0.5	3	2	3	2	2	1	2	0	0
1	2	1	3	1	1	1	1	0	0
2	2	1	1	1	1	0	1	0	0
NAA									
0.05	1	1	1	2	0	0	2	0	0
0.1	2	3	3	2	1	2	2	0	0
0.5	2	2	3	2	1	2	1	0	0
1	2	2	2	1	1	1	1	0	0
2	1	1	1	1	0	0	1	0	0
IAA									
0.05	2	1	1	1	0	0	1	0	0
0.1	2	2	1	1	0	0	1	0	0
0.5	3	2	2	2	0	1	2	0	0
1	3	2	3	3	0	2	3	0	0
2	1	1	1	2	0	0	2	0	0

0: nothing, 1: very low, 2: low, 3: medium, 4: high, 5: very high.

Table2: The Effect of explants Source and different cytokinin on callus formation and organogenesis *in Prosopis koelziana* Burkart

PGR	Meristem			hypocotyl			leaves		
	callus	shoot	root	callus	shoot	root	callus	shoot	root
BAP									
0.05	1	0	1	1	0	0	1	0	0
0.1	1	1	1	2	0	0	1	0	0
0.5	2	3	0	2	1	0	2	0	0
1	3	3	0	2	1	0	2	0	0
2	2	2	0	2	1	0	1	0	0
2iP									
0.05	1	1	0	1	0	0	1	0	0
0.1	1	1	0	1	0	0	1	0	0
0.5	2	2	0	2	0	0	2	0	0
1	2	2	0	2	1	0	2	0	0
2	3	3	0	3	1	0	2	0	0
Kin									
0.05	0	0	0	1	0	0	1	0	0
0.1	1	1	1	1	0	0	1	0	0
0.5	2	1	1	2	1	0	2	0	0
1	2	2	0	3	1	0	2	0	0
2	1	1	0	2	0	0	2	0	0

0: nothing, 1: very low, 2: low, 3: medium, 4: high, 5: very high.

Table3: The Effect of explant Source, BAP and NAA on callus formation and organogenesis *in Prosopis koelziana* Burkart

Explant source		Meristem			hypocotyl			Leaves		
BAPmgl^{-1}	NAAmgl^{-1}	callus	shoot	root	callus	shoot	root	callus	shoot	root
0.5	0.05	3	3	0	4	2	0	3	0	0
0.5	0.1	2	2	1	2	1	2	3	0	0
0.5	0.5	1	0	1	1	0	1	1	0	0
0.5	1	2	0	3	1	0	2	1	0	0
1	0.05	3	3	0	2	2	0	3	0	0
1	0.1	5	5	1	3	2	0	4	0	0
1	0.5	2	2	1	1	1	1	2	0	0
1	1	1	0	0	1	0	0	1	0	0
2	0.05	2	3	0	2	1	0	2	0	0
2	0.1	2	1	0	3	1	1	3	0	0
2	0.5	2	0	0	1	1	1	2	0	0
2	1	1	0	0	1	1	0	1	0	0

0: nothing, 1: very low, 2: low, 3: medium, 4: high, 5: very high.

Table 4: The effect of explants Source and different concentrations of NaCl on callus formation and shoot regeneration *in Prosopis koelziana* Burkart

-Explant source	Meristem		hypocotyl		Leaves	
NaCl gl^{-1}	callus	shoot	callus	shoot	callus	shoot
0	5	5	4	2	4	0
2	5	5	4	2	4	0
4	5	5	3	1	4	0
6	3	2	3	0	2	0
8	2	1	2	0	1	0
10	1	0	1	0	0	0
12	0	0	0	0	0	0
14	0	0	0	0	0	0

0: nothing, 1: very low, 2: low, 3: medium, 4: high, 5: very high.

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پاسخ گیاه کهور (***Prosopis koelziana Burkart***) در کشت در شیشه و تنش شوری

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چکیده

در این پژوهش اثر منبع جداکشت، تنظیم کننده‌های رشد و NaCl بر اندام‌زایی و تجمع پرولین در گیاه کهور از گونه‌ای بردبار به شوری مورد مطالعه قرار گرفته است. مریستم، برگ جوان ولپه از دانه‌رست‌های کشت شده در شیشه جدا شده است. سپس جدا کشت‌ها در محیط MS به اضافه تنظیم کننده‌های مختلف رشد کشت شدند. جداکشت‌های مریستمی چندین اندام هوایی و ریشه تولید نمودند. برعکس کالوس‌های برگی اندام هوایی و ریشه تولید نکردند. بهترین تیمار برای ایجاد اندام هوایی محیط کشت محتوی یک میلی‌گرم در لیتر بنزیل آمینوپورین و ۰/۱ میلی‌گرم در لیتر نفتالن استیک بود. اکسین تولید کالوس و ریشه را تحریک نمود، برعکس سیتوکینین تشکیل اندام هوایی را تسریع نمود. اثر غلظت‌های مختلف NaCl (۰، ۲، ۴، ۶، ۸، ۱۰گرم در لیتر) بر اندام‌زایی و تجمع پرولین نیز مورد بررسی قرار گرفت. نتایج نشان داد که شوری موجب کاهش اندام‌زایی شد، ولی اثر کمی در القاء کالوس داشته است. گرچه غلظت بالای شوری موجب بازدارندگی تشکیل کالوس و اندام‌زایی شد. محتوای پرولین بطور معنی‌داری با افزایش شوری افزایش یافت، ولی افزایش NaCl از ۸ میلی‌گرم در لیتر تا ۱۰ میلی‌گرم در لیتر محتوای پرولین را اضافه نکرد.

واژه‌های کلیدی: منبع جداکشت، تنظیم کننده‌های رشد، اندام‌زایی، کالوس، پرولین، کهور