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Research Article

IN VITRO REGENERATION THROUGH ORGANOGENESIS AND EMBRYOGENESIS IN POMEGRANATE CULTIVAR BHAGAWA

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Abstract: Rapid *in vitro* propagation of pomegranate in Bhagava cultivar using organogenesis and embryogenesis is conducted.MS basal media along with BAP(1.5mg/L.) has given maximum success in shoot induction. Use of Silver nitrate(0.5mg/it.) along with Adenine sulphate (25mg/L) has shown rapid increase in shoot length while use of BAP (1.0mg/L) and kinetin(1.0mg/L) has given maximum number of multiple shoots. In another experiment, embryogenic callus was developed from explants on MS with BAP (0.8mg/L and 1.0mg/L) .The shoots were obtained from callus using BAP(1.0mg/L), Kinetin (2.0mg/L) and NAA(0.1mg/L). Plant growth was rapid in organogenesis while embryogenesis has responded with more number of multiple shoots and rapid plant growth. Both organogenesis and embryogenesis have resulted in satisfactory rooting and hardening percentage of 80-90%. These results indicate feasibility of using embryogenesis or organogenesis for rapid commercial *in vitro* propagation in pomegranate.

Keywords: Pomegranate, organogenesis, embryogenesis, silver nitrate, BAP

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Introduction

Traditional method of pomegranate propagation through hard wood cuttings requires atleast one year for the plant establishment. Besides it is prone to several soil born and systemic viral, fungal and bacterial infections. It is also not possible to obtain uniform yield and quality [1]. To address all these limitations propagation of pomegranate through tissue culture is advantageous. The constrains in obtaining desired number of plantlets at less cost through tissue culture require standardization and modification of protocols for elite cultivars of this important crop [2,3]. We are reporting here two methods for rapid *in vitro* propagation of pomegranate using organogenic and embryogenic approaches respectively.

Materials and Methods

Plant material

The explants were obtained from mother culture of Bhagava cultivar maintained in the commercial green house of the Lokmangal Organics and Research, Pvt Ltd (LORDS), Wadala, Solapur which has procured the plants from ICAR-NRCP, Solapur. The shoots, nodal segments, roots and apical meristem were screened in initial experiments and it was found that nodal segments have shown maximum regeneration potential. The explants excised from disease and virus free plants were surface sterilized and inoculated on M.S. basal media with hormonal and specific additional components' additions as described in the results [4]. All the cultures were maintained in the R&D facility of LORDS, Solapur at 25(+/-2)^{0}C and 16hrs./8hrs.light /dark photoperiod except where separate conditions are needed. The observations were recorded in a standard format applying statistical methods for randomization and reduction of manual and experimental errors. Each experiment was repeated twice and average results for at least 20 explants in each experimental parameter were recorded and used for analysis [5].

Results

Shoot Induction

As shown in the Table below [Table-1] shoot induction was observed in all pomegranate nodal segments used as explants after average 21days from inoculation:

Table-1 Effect of BAP on shoot induction in pomegranate

SN	Medium	BAP (mg/L)	No. of shoots formed	Average Shoot length (cm)
1	MS + BAP	0.5	1	0.5
2	MS + BAP	1.0	1	0.75
3	MS + BAP	1.5	2	1
4	MS + BAP	2.0	1	0.75

Shoot multiplication

The hormone Adenine sulphate (15mg/L, 20mg/L, 25mg/L) and $AgNO_3$ (0.5mg/L, 1.0mg/L, 1.5, mg/L) or BAP and /or kinetin were used for multiple shoot formation along with M.S. basal media. The results are as shown in the [Table-2]

Root induction and hardening

IBA (0.5mg/L & 1.0 mg/L) was used for root induction. Roots were developed in 10-12 days in both combinations. Primary hardening for 7-10 days was followed by secondary hardening on sterile cocopeat, farm yard manure and micronutrient mix (1:1:1). The hardened plants were transferred to greenhouse. Our results indicate average 72.8 % of survival at hardening stage [6-8].

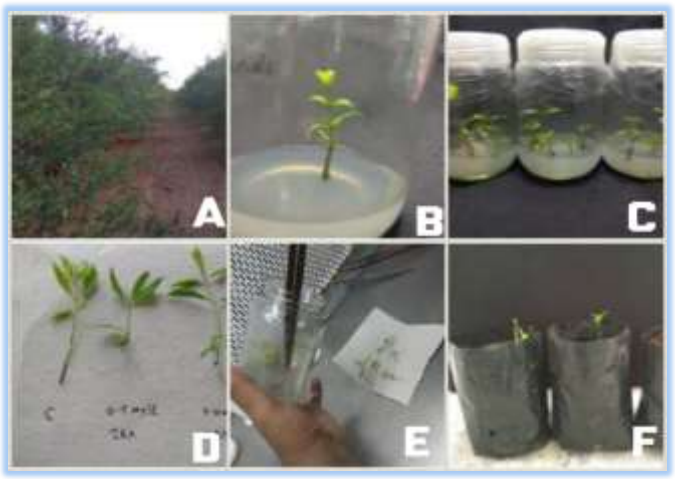

Fig-1 Propagation of pomegranate *cv*. Bhagava through embryogenesis (A:Pomegranate mother orchard variety Bhagava; B:Shoot induction; C:Shoot multiplication; D:Root formation; E &F : Hardening)

Table-2 Effect of Adenine sulphate, Silver Nitrate, kinetin and BAP on shoot multiplication in pomegranate

SN	Medium	Adenine sulphate + $AgNO_3$	No of shoot/ explant	Shoot length (cm)
1	MS +adenine suiphate+$AgNO_3$	15 mg/L+0.5 mg/L	0.7	1.8
2	MS +adenine suiphate+$AgNO_3$	20 mg/L+1.0 mg/L	0.8	2.8
3	MS +adenine suiphate+$AgNO_3$	25 mg/L+1.5 mg/L	1	1.7
4	MS + Kn + BAP	1.0mg/L+1.0 mg/L	4.5	1.5
5	MS +BAP	0.5 mg/L	1	5

Embryogenesis: There are limited reports of embryogenesis and plant regeneration from embryogenesis in pomegranate [9]. We have used MS media with BAP(0.4mg/L,0.6 mg/L,0.8 mg/L and 1.0 mg/L) . After repeated subculturing for 4 weeks proliferating embryogenic calli were observed [Table-3].

Table-3 Embryogenic callus formation in pomegranate shoot explants on MS media with BAP

SN	Media	Observation (After 4 weeks of culturing)
1	M.S+BAP(0.4mg/L)	No callus formation
2	M.S+BAP(0.6mg/L)	No callus formation
3	M.S+BAP(0.8mg/L)	Proliferating green coloured, callus formation, confirmed embryogenesis through microscopy
4	M.S+BAP(1.0mg/L)	Proliferating green coloured, callus formation, confirmed embryogenesis through microscopy

Rediferentiation: After 1-week countable number of shoots were developed from embryogenic calli kept on following combinations of hormones [Table-4].

Root induction and hardening: The plants were transferred on half strength MS media containing NAA (0.5mg/L and 1.0mg/L). Roots were developed in 1 week on all the explants. After 3 weeks of hardening the plants were transferred to greenhouse [9,10].

Table-4 Redifferentiation of embryogenic callus into shoots in pomegranate

SN	MS medium supplemented with			Average No. of shoots	Average Shoot Length (cm)	
	NAA (mg/L)	Kinetin (mg/L)	BAP (mg/L)		1 week	2 weeks
1	0.05	1.0	1.0	1.0	0.5	1.1
2	0.05	2.0	0.5	2.0	0.5	2.4
3	0.1	2.0	1.0	2.5	0.5	1.6

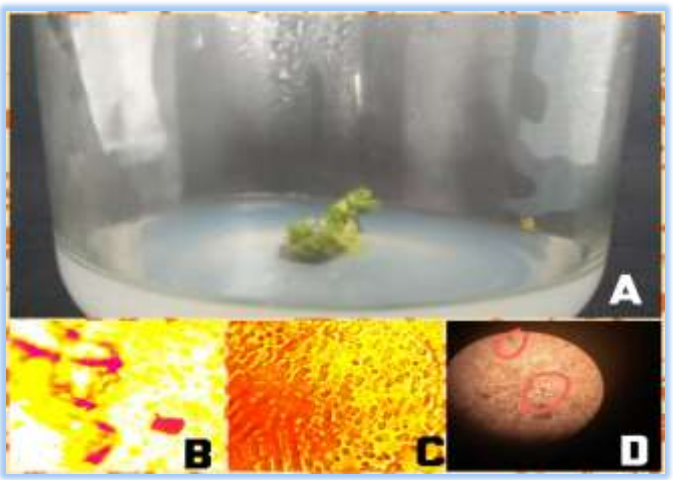

Fig-2 Development of embryogenic callus and plantlet in pomegranate (A:Redifferentiation of embryogenic callus into shoots, B,C&D Embryogenic cells through different stages of development observed under microscope(magnification of 400x) after staining with Acetocarmine)

Discussion

Shoot multiplication: Patil *et al.,* have obtained multiple shoots using Adenine sulphate and silver nitrate on WPM. As shown above we have obtained the results showing rapid increment in shoot length when silver nitrate was used as one of the components. Kinetin(1.0mg/l) and BAP(1.0mg/l) have given maximum number of shoots [6-8]. As shown in the table 2 our results in Bhagva variety have shown rapid growth of plant length using adenine sulphate and silver nitrate as additional components. Use of kinetin along with BAP has given multiple shoots emphasizing use of kinetin as one of the components can produce multiple shoots in pomegranate which are difficult to obtain otherwise [7,8]. As shown in [Table-4] shoot formation was observed in all the combinations studied. Maximum number of shoots were obtained in NAA 0.1 mg/L+ Kn 2.0 mg/L+ 1.0 mg/L BAP. These results indicate successful use of embryogenesis in rapid proliferation of pomegranate from callus through embryogenesis. The shoot length was 1.5cm within two weeks. After subculturing on same combinations, the shoot length increased rapidly within one week in Sr.No.2 while multiple shoots were obtained in Sr.No.3 [9-11].

Conclusion

Use of organogenesis and embryogenesis was successful in obtaining *in vitro* shoots in Bhagava variety of pomegranate but embryogenesis has given rapid response compared to organogenesis. There were no differences in rooting and hardening percentages as well as plant growth.

Application of research

After successful organogenesis and embryogenesis our next goal is to study genetic fidelity mainly in the embryogenic plants. Another objective for further research is to use embryogenesis method for commercial *in vitro* propagation of Bhagava cultivar using liquid media.

Research Category: Plant Tissue Culture and Embryogenesis

Abbreviations:

BAP: 6-Benzylaminopurine
MS: Murashige and Skoog basal Media
NAA: Naphtalene Acetic Acid
$AgNO_3$: Silver Nitrate

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