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SEASONAL VARIATION IN THE SHOOT-REGENERATION POTENTIAL OF THE NODAL EXPLANTS OF *ROSA SETIGERA*

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The present paper reports seasonal variation in the regeneration potential of nodal explant of *Rosa setigera* over MS medium. We evaluated the shoot regeneration potential of the explants harvested from mature plants viz. shoot-tip, nodal segment (1.5cm), internodal segment (1.5cm) and leaf disc. The explants were harvested in rainy, winter and summer seasons and inoculated over MS medium fortified with various combinations of NAA, 2,4-D, BAP and kinetin. Among the explants, only nodal segments harvested in rainy and winter seasons induced microshoots. The response of explant varied with the concentration and combination of plant growth regulators and the season of harvest. The best response, in terms of multiple shoot induction, by nodal explants was exhibited over the medium fortified with 2.2M BAP in rainy season and 0.5M NAA + 8.8M BAP in winter season.

Keywords: floriculture; MS medium; PGR; rose; seasonal effect

Roses are well-known world-over for their aesthetic value. They find place in almost all the gardens in the regions where they could be grown. The flower of rose is famous for its shape, size, colour and fragrance. Numerous horticultural varieties of roses are available for planting (Kanchanapoom *et al*. 2009). Roses belong to the genus *Rosa*, which has 150 species and over 1,400 cultivars (Jaskani *et al*. 2005). Amongst the numerous species of rose is *Rosa setigera* Michx. It is a perennial shrub commonly grown in public gardens under many horticultural forms (Naik 1998).

Rose oil has diverse uses. For example it is used in soap and cosmetics for its aroma. Similarly, rose oil is added to tea and beverages as flavouring agent. Rose oil also has antihystological, gall-curative, antispasmodic and relaxing action (Khan and Shoaib-Ur-Rehman 2005, Loghmani-Khouzani *et al.* 2007). Water extract of rose possesses anti-inflammatory, analgesic, antibacterial, antiviral and antifungal effects (Vinokur *et al.* 2006). Rose petals are also used to make rose wine, rose marmalade, rose jam, rose honey and extraction of vitamin C from heps for medicinal uses (Khan *et al*. 2007). Rose also has the significant importance in traditional medicines. Pharmacological effects of this plant on premenstrual breast tenderness and reduction of inflammation, especially of the neck, have been reported (Loghmani-Khouzani *et al*. 2007).

Although, roses can be propagated through budding and grafting but these methods are of less use when *in vitro* mutagenesis and genetic engineering are used for crop improvement. Both these modes of crop improvement rely on an efficient regeneration protocol to ensure maximum production of plants (Khan *et al*. 2009). Need for micropropagating heterozygous species of rose like *R. chinensis* has been pressed by Bharadwaj *et al.* (2006). This is necessary for production of true-to-type progenies of a variety. Numerous reports about the micropropagation of various species of roses are available (Rout *et al.* 1999, Jha 2004; Pati *et al.* 2006, Canli and Kazaz 2009). *In vitro* propagation and root induction was reported by Elliot (1970) in *R. multiflora*. Ma *et al.* (1996) have reported the *in vitro* regeneration of *R. setigera*. The microshoots were induced using 8.8M BAP (Benzylaminopurine) and rooted with 11.4M IAA (Indole acetic acid).

Various factors like PGRs (plant growth regulators) (Bressan *et al.* 1982), agar concentration in medium (Ghashghaie *et al*. 1991) and position of explant on the branch (Shirdel *et al*. 2013) affect the shoot regeneration. Moreover, species-dependent

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difference in the regeneration of explants of rose has also been reported. Khosh-Kui and Sink (1982) reported higher regeneration frequency of *R. damascena* as compared to *R. canina*. The response of an explant over its medium depends on the intrinsic and extrinsic PGR concentration (Badere *et al*. 2002). It is well known that the environmental conditions besides the developmental stage of plant affects intrinsic levels of PGRs. The commercial production of micropropagated rose plants would need year round supply of explants. Thus, a study was planned to evaluate the seasonal variation in the regeneration potential of explants of *R. setigera*. This would be of help in developing a cost-efficient regeneration strategy for rose micropropagation.

MATERIALS AND METHODS

Harvesting the explants

The explants viz. shoot-tip, nodal (1.5cm) & internodal (1.5cm) segments (4^{th} to 8^{th} node from apex) and leaf disc (6mm) were harvested from the plant in rainy (July- October), winter (November-February) and summer (March-June) seasons. For this juvenile shoots with 9-10 nodes were harvested from full grown plant. Each shoot after harvesting, was dipped in cold water until used.

Surface sterilization

The explants were washed with distilled water containing 5 drops of Extran for 10min. It was followed by 3 washes of autoclaved distilled water for 5min each. Later the explants were processed inside the laminar airflow hood. First, the explants were washed with 5% sodium hypochlorite containing 2 drops of Tween-20 for 10min. It was followed by 3 washes of autoclaved distilled water for 5min each. Later on the explants were washed with 70% ethanol for 5min and again followed by 3 washes of autoclaved distilled water for 5min each.

Inoculation and incubation of explants

The surface sterilized explants (25 explants each per medium) were then inoculated over the MS medium (Murashige and Skoog 1962) fortified with various concentrations and combinations of PGRs. The PGRs used were BAP (2.2, 4.4, 6.6 & 8.8µM), kinetin (2.3, 4.6, 6.9 & 9.2µM), NAA (0.0, 0.5, 1.0 & 2.5µM) and 2,4-D (0.0, 0.4, 0.8 & 2.0µM). Culture vessels containing inoculated explants were incubated at 25±2º C and 16h photoperiod. The response of explants over media was monitored on weekly basis. Explants which induced at least one shoot were scored as responding explants. These data were then used to calculate the frequency of response and number of shoots per explant. Later these two values were multiplied to derive the regeneration efficiency.

Statistical analysis

The mean and standard error for number of shoots per explant was computed in the present investigation. Later, the significance of the effect of PGRs on shoot per explant was derived by performing the Duncan's multiple range test (DMRT). Similarly, the effect of season and PGR on regeneration efficiency was tested using one-way and two-way ANOVA. The statistical computations were done with the help of MS-Excel, XL-STAT and Graphpad Prism.

RESULTS AND DISCUSSION

Amongst all the explants used, only nodal segment harvested in rainy and winter seasons responded by forming the shoots (Fig. 1). Shoot-tip, leaf disc and internode did not respond at all, irrespective of the season. Similarly, nodal segment harvested in summer season also did not respond over any of the media tested (Data not shown). There is a variation in the regeneration potential of each explant. Some explants are amenable to regeneration, while others are not. Thus, the choice of explant is very crucial for micropropagation of plant. The nodal segments have been used for mass production of the plants (Rout *et al.* 1999, Zaki *et al.* 2011). Nodal segments also show earlier response than the other explants. It may be due to presence of better nutrient translocation and

endogenous level of PGRs in the nodal segment(Hsia and Korban 1996, Maurya *et al.* 2013). However,Ma *et al.* (1996) found that the nodes from the medial part of the branch responded efficiently in *R. rugosa*, *R. wichuraiana*, *R. setigera*, *R. laevigata*, *R. banksiae*, *R. roxburghii*, *R. fortuniana*. Shirdel *et al.* (2013) also found similar results in *R. canina* where the nodes from central part of the branches developed proliferating shoots. Even during the present investigation we found that 4th to 8th node from shoot apex only induced shoot(s) over the medium.

Nodal explants formed mostly 1.0 shoot per explant with the frequency between 28 and 100%, depending upon the season of harvesting and the PGR concentration and combination. Kinetin when combined with either NAA or 2,4-D induced 1.0 shoot per explant in the explants harvested in rainy and winter seasons. The only exception to this was the nodal segment harvested in winter season inoculated over the media containing 0.5µM NAA and 9.2µM kinetin where it formed 1.17 shoots per explant with the frequency of 72% (Tables 1-4).

The nodal segments harvested in rainy and winter season responded differentially the medium containing BAP with either NAA or 2,4-D. While, NAA at higher concentrations seem to inhibit the induction of multiple shoots; 2,4-D promoted it (Tables 1-4). BAP alone induced upto 1.14 shoots per explant at 8.8µM concentration with the frequency of 40% in the explants harvested in rainy season. But, the effect of 8.8µM BAP was slightly enhanced when combined with 0.5µM NAA, where 1.20 shoots per explant with the frequency of 80% were induced (Table 1). In contrast to this, the explants harvested in winter season responded well over the media containing 1.0 or 2.5µM NAA. The maximum shoots per explant induced were 1.63 with the frequency of 76% over the media fortified with

Table 1. Response of nodal explant of *Rosa setigera* over MS media fortified with NAA & BAP.

Concentration (µM)		Rainy Season			Winter Season		
NAA	**BAP**	**S/E***	**F (%)***	**Efficiency***	**S/E***	**F (%)***	**Efficiency***
0.0	2.2	$1.12^{a} \pm 0.6$	100	112	$1.00^{bcd} \pm 0.4$	84	84
0.0	4.4	$1.00^{bcde} \pm 0.4$	76	76	$1.00^{bcd} \pm 0.2$	92	92
0.0	6.6	$1.12^{a} \pm 0.7$	96	108	$1.00^{bcd} \pm 0.5$	84	84
0.0	8.8	$1.14^{cdef} \pm 0.6$	40	46	$1.00^{cd} \pm 0.4$	76	76
0.0	11.0	$1.00^{bcde} \pm 0.5$	72	72	$1.14^{bcd} \pm 0.2$	84	96
0.5	2.2	$1.00^{def} \pm 0.4$	52	52	$1.00^{e} \pm 0.4$	20	20
0.5	4.4	$1.16^{abcd} \pm 0.4$	72	84	$1.00^{bcd} \pm 0.4$	84	84
0.5	6.6	$1.00^{abc} \pm 0.6$	88	88	$1.00^{d} \pm 0.6$	68	68
0.5	8.8	$1.20^{ab} \pm 1.0$	80	96	$1.24^{a} \pm 0.6$	100	124
0.5	11.0	$1.00^{def} \pm 0.6$	52	52	$1.00^{cd} \pm 0.4$	76	76
1.0	2.2	$1.00^{ab} \pm 0.2$	92	92	$1.63^{a} \pm 0.5$	76	124
1.0	4.4	$1.00^{cdef} \pm 0.7$	56	56	$1.00^{bcd} \pm 0.6$	88	88
1.0	6.6	$1.00^{abcd} \pm 0.4$	84	84	$1.17^{ab} \pm 0.4$	92	108
1.0	8.8	$1.00^{f} \pm 0.4$	32	32	$1.00^{e} \pm 0.3$	20	20
1.0	11.0	$1.00^{f} \pm 0.4$	28	28	$1.00^{d} \pm 0.5$	68	68
2.5	2.2	$1.00^{abc} \pm 0.4$	88	88	$1.00^{abc} \pm 0.0$	100	100
2.5	4.4	$1.20^{ab} \pm 0.6$	80	96	$1.24^{a} \pm 0.5$	100	124
2.5	6.6	$1.00^{ef} \pm 0.4$	44	44	$1.00^{bcd} \pm 0.4$	88	88
2.5	8.8	$1.00^{f} \pm 0.2$	32	32	$1.00^{bcd} \pm 0.6$	84	84
2.5	11.0	$1.00^{f} \pm 0.7$	28	28	$1.00^{bcd} \pm 0.4$	88	88

Mean with same letter(s) in the same column are not significantly different at 5% using Duncan's multiple range test.

The response of explants harvested in summer was nil.

*Abbreviations: S/E = Number of shoots/explant, F = % Frequency, Efficiency = Regeneration efficiency (S/E x F)

[Same for table no. 2, 3 & 4]

Table 2. Response of nodal explant of *Rosa setigera* over MS media fortified with NAA & kinetin.

Concentration (µM)		Rainy Season			Winter Season		
NAA	**kinetin**	**S/E**	**F (%)**	**Efficiency**	**S/E**	**F (%)**	**Efficiency**
0.0	2.3	$1.00^{abcde} \pm 0.4$	64	64	$1.00^{bcde} \pm 0.4$	68	68
0.0	4.6	$1.00^{abcd} \pm 0.5$	72	72	$1.00^{abcde} \pm 0.3$	80	80
0.0	6.9	$1.00^{bcde} \pm 0.6$	40	40	$1.00^{de} \pm 0.4$	60	60
0.0	9.2	$1.00^{bcde} \pm 0.6$	44	44	$1.00^{abcde} \pm 0.6$	76	76
0.0	11.5	$1.00^{e} \pm 0.7$	28	28	$1.00^{abcde} \pm 0.3$	80	80
0.5	2.3	$1.00^{bcde} \pm 0.5$	40	40	$1.00^{abcde} \pm 0.5$	72	72
0.5	4.6	$1.00^{bcde} \pm 1.0$	44	44	$1.00^{abcde} \pm 0.4$	76	76
0.5	6.9	$1.00^{bcde} \pm 0.7$	48	48	$1.00^{ab} \pm 0.2$	92	92
0.5	9.2	$1.00^{abcde} \pm 0.9$	64	64	$1.17^{abcd} \pm 0.4$	72	84
0.5	11.5	$1.00^{cde} \pm 0.4$	36	36	$1.00^{abcde} \pm 0.2$	72	72
1.0	2.3	$1.00^{abcde} \pm 0.4$	60	60	$1.00^{abcde} \pm 0.4$	76	76
1.0	4.6	$1.00^{e} \pm 0.9$	28	28	$1.00^{cde} \pm 0.4$	64	64
1.0	6.9	$1.00^{cde} \pm 0.7$	36	36	$1.00^{bcde} \pm 0.2$	68	68
1.0	9.2	$1.00^{de} \pm 0.7$	32	32	$1.00^{abcde} \pm 0.4$	72	72
1.0	11.5	$1.00^{cde} \pm 0.9$	36	36	$1.00^{e} \pm 0.4$	56	56
2.5	2.3	$1.00^{a} \pm 0.2$	96	96	$1.00^{a} \pm 0.2$	96	96
2.5	4.6	$1.00^{ab} \pm 0.3$	80	80	$1.00^{a} \pm 0.2$	96	96
2.5	6.9	$1.00^{a} \pm 0.2$	92	92	$1.00^{abc} \pm 0.4$	88	88
2.5	9.2	$1.00^{abc} \pm 0.4$	76	76	$1.00^{abcde} \pm 0.7$	76	76
2.5	11.5	$1.00^{abcde} \pm 0.5$	74	74	$1.00^{abcd} \pm 0.4$	84	84

Table 3. Response of nodal explant of rose over MS media fortified with 2,4-D & BAP

Concentration (µM)		Rainy Season			Winter Season		
2,4-D	**BAP**	**S/E**	**F (%)**	**Efficiency**	**S/E**	**F (%)**	**Efficiency**
0.0	2.2	$1.12^{a} \pm 0.4$	100	112	$1.00^{abcde} \pm 0.6$	84	84
0.0	4.4	$1.00^{abcde} \pm 0.7$	76	76	$1.00^{abc} \pm 0.4$	92	92
0.0	6.6	$1.12^{a} \pm 0.6$	96	108	$1.00^{abcd} \pm 0.4$	84	84
0.0	8.8	$1.14^{de} \pm 1.2$	40	46	$1.00^{bcd} \pm 0.2$	76	76
0.0	11.0	$1.00^{bcde} \pm 0.5$	72	72	$1.00^{abc} \pm 0.2$	84	84
0.4	2.2	$1.00^{abcd} \pm 0.3$	80	80	$1.26^{abc} \pm 0.2$	76	96
0.4	4.4	$1.00^{abcd} \pm 0.4$	84	84	$1.00^{cd} \pm 0.8$	64	64
0.4	6.6	$1.00^{ab} \pm 0.0$	100	100	$1.00^{bcd} \pm 0.4$	72	72
0.4	8.8	$1.00^{abcde} \pm 0.7$	76	76	$1.25^{cd} \pm 0.7$	48	60
0.4	11.0	$1.00^{cde} \pm 0.3$	60	60	$1.25^{abcd} \pm 0.7$	64	80
0.8	2.2	$1.00^{cde} \pm 0.8$	60	60	$1.00^{abcd} \pm 0.4$	88	88
0.8	4.4	$1.00^{e} \pm 0.6$	40	40	$1.26^{a} \pm 0.7$	92	116
0.8	6.6	$1.00^{abcde} \pm 0.6$	76	76	$1.00^{cd} \pm 0.5$	64	64
0.8	8.8	$1.00^{abc} \pm 0.2$	96	96	$1.00^{d} \pm 0.8$	52	52
0.8	11.0	$1.00^{abcde} \pm 0.6$	76	76	$1.00^{cd} \pm 0.5$	60	60
2.0	2.2	$1.00^{abc} \pm 0.2$	96	96	$1.30^{ab} \pm 0.2$	80	104
2.0	4.4	$1.00^{abcd} \pm 0.3$	80	80	$1.18^{abcd} \pm 0.3$	68	80
2.0	6.6	$1.00^{abcd} \pm 0.4$	92	92	$1.00^{cd} \pm 1.0$	64	64
2.0	8.8	$1.00^{abcde} \pm 0.2$	76	76	$1.28^{abc} \pm 0.4$	72	92
2.0	11.0	$1.00^{bcde} \pm 0.5$	74	74	$1.16^{abcd} \pm 0.5$	76	88

Table 4. Response of nodal explants of rose over MS media fortified with 2,4-D & kinetin.

Concentration (µM)		Rainy Season			Winter Season		
2,4-D	kinetin	S/E	F (%)	Efficiency	S/E	F (%)	Efficiency
0.0	2.3	$1.00^{abc} \pm 0.2$	96	96	$1.00^{a} \pm 0.7$	68	68
0.0	4.6	$1.00^{abcde} \pm 0.3$	80	80	$1.00^{a} \pm 0.5$	80	80
0.0	6.9	$1.00^{abcd} \pm 0.2$	92	92	$1.00^{a} \pm 0.4$	60	60
0.0	9.2	$1.00^{abcde} \pm 0.2$	76	76	$1.00^{a} \pm 0.4$	76	76
0.0	11.5	$1.00^{abcde} \pm 0.2$	74	74	$1.00^{a} \pm 0.6$	80	80
0.4	2.3	$1.00^{abcde} \pm 0.3$	80	80	$1.00^{a} \pm 0.4$	72	72
0.4	4.6	$1.00^{abcde} \pm 0.4$	84	84	$1.00^{a} \pm 0.9$	64	64
0.4	6.9	$1.00^{ab} \pm 0.2$	96	96	$1.00^{a} \pm 0.5$	68	68
0.4	9.2	$1.00^{abcde} \pm 0.4$	84	84	$1.00^{a} \pm 0.4$	76	76
0.4	11.5	$1.00^{abcd} \pm 0.2$	88	88	$1.00^{a} \pm 0.5$	80	80
0.8	2.3	$1.00^{c} \pm 0.8$	60	60	$1.00^{a} \pm 0.7$	72	72
0.8	4.6	$1.00^{a} \pm 0.0$	100	100	$1.00^{a} \pm 0.4$	76	76
0.8	6.9	$1.00^{abcde} \pm 0.4$	84	84	$1.00^{a} \pm 0.7$	72	72
0.8	9.2	$1.00^{abcd} \pm 0.2$	92	92	$1.00^{a} \pm 0.4$	84	84
0.8	11.5	$1.00^{bde} \pm 0.9$	68	68	$1.00^{a} \pm 0.2$	68	68
2.0	2.3	$1.00^{abcde} \pm 0.4$	84	84	$1.00^{a} \pm 0.5$	76	76
2.0	4.6	$1.00^{abcd} \pm 0.2$	88	88	$1.00^{a} \pm 0.2$	88	88
2.0	6.9	$1.00^{abcde} \pm 0.4$	84	84	$1.00^{a} \pm 0.4$	84	84
2.0	9.2	$1.00^{abcde} \pm 0.3$	80	80	$1.00^{a} \pm 0.4$	68	68
2.0	11.5	$1.00^{f} \pm 0.7$	28	28	$1.00^{a} \pm 0.5$	60	60

Table 5. One way ANOVA for regeneration efficiency over two seasons (rainy and winter).

Source	SS	df	MS	F value
Between seasons	3256	1	3256	7.27*
Within season	61779	138	448	
Total	65035			

*Value significant at 5% level of confidence.

Table 6. Two way ANOVA for regeneration efficiency over two seasons (rainy and winter) and BAP at various concentrations with 2.5M NAA.

Source	SS	df	MS	F value
2.5mM NAA + BAP	4442	4	1110	5.98
Season	3842	1	3842	20.70*
Error	742	4	186	
Total	9026			

*Value significant at 5% level of confidence.

Table 7. Two way ANOVA for regeneration efficiency over two seasons (rainy and winter) and kinetin at various concentrations with 0.4M 2,4-D.

Source	SS	df	MS	F value
0.4mM 2,4-D + Kinetin	138	4	34	0.81
Season	518	1	518	12.23*
Error	170	4	42	
Total	826			

*Value significant at 5% level of confidence.

Table 8. Two way ANOVA for regeneration efficiency over two seasons (rainy and winter) and kinetin at various concentrations with 0.5M NAA.

Source	SS	df	MS	F value
0.5mM NAA + Kinetin	406	4	102	1.12
Season	2310	1	2310	25.56**
Error	362	4	90	
Total	3078			

**Value significant at 1% level of confidence.

Table 9. Two way ANOVA for regeneration efficiency over two seasons (rainy and winter) and kinetin at various concentrations with 1.0M NAA

Source	SS	df	MS	F value
1.0mM NAA + Kinetin	650	4	162	3.03
Season	2074	1	2074	38.69**
Error	214	4	54	
Total	2938			

**Value significant at 1% level of confidence.

1.0 and 2.2µM BAP. Moreover, the higher concentration of NAA also increased the percent frequency of response of the explants harvested in the winter season (Table 1). NAA with kinetin induced 1.0 shoot per explant in the nodal segments harvested in rainy season with the frequency ranging from 28 to 96%. The same combination, however, improved the frequency of response in the explants harvested in winter season. The frequency in this case varied between 56 to 96% (Table 2).

A differential effect of BAP with 2,4-D was evident over the explants harvested in rainy and winter season. In the presence of 2,4-D, BAP lowered the number of shoots per explant to 1.0 in the explants harvested in rainy season. However, the effect of BAP on the explants harvested in winter season improved when 2,4-D was present in the medium. In the explants harvested in rainy season, 8.8µM BAP induced maximum number of shoots per explant i.e. 1.14 with the frequency of 40%. Similarly, the explants harvested in winter season responded maximum over the media containing 2.0µM 2,4-D with 2.2µM BAP by forming 1.30 shoots per explant with the frequency of 80%. In general, higher concentration of 2,4-D with BAP favoured the induction of multiple shoots (Table 3). Kinetin with 2,4-D induced 1.0 shoot per explant irrespective of the season of harvest. The explants harvested in rainy season responded to the media containing kinetin and 2,4-D at the frequency between 28 and 100%. Similarly, the regeneration frequency of explants harvested in winter season was from 60 to 88% over the medium containing 2,4-D and kinetin (Table 4).

Later we performed one-way ANOVA using season as a factor. The analysis revealed a significant difference (at 5% level of confidence) in the response of explants harvested during summer and winter season (Table 5). Subsequently, to have a precise idea about differential effect of season, we performed two-way ANOVA using season and PGR combination as the factors. In this analysis we found season to have differential effect on shoot regeneration when the explants were inoculated over the medium with certain specific PGR combination. The differential effect of seasons was significant at 5% level of confidence over the medium fortified with either 2.5M NAA with BAP (Table 6) or 0.4M 2,4-D with kinetin (Table 7). On the other hand when the medium was fortified with either 0.5M NAA with kinetin (Table 8) or 1.0M NAA with kinetin (Table 9) the differential effect of season was significant at 1% level of confidence.

Season-dependent collection of explants is very crucial for *in vitro* culture (Lee *et al*. 2010). In mature plant, different seasons may influence the internal physiological state of the explants which may influence the outcome of aseptic culture (Kumar *et al*. 2005, Kartsonas

and Papafotiou 2007). It is well known that the response of explants over the media depends upon the exogenous and endogenous PGR concentration (Badere *et al*. 2002). So, the response of same explant over the same medium can vary in different season due to variation in the endogenous PGR levels. Roy *et al.* (2004) too found winter to be the better season to harvest explants for shoot regeneration in rose. Barna and Wakhlu (1995) and Kumar *et al.* (2001) suggested collection of explants of *R. damascena* between September to October and in early spring for better regeneration. Bharadwaj *et al.* (2006) made the similar recommendation in case of shoot regeneration in *R. chinensis*. Ozel and Arslan (2006) in Turkey also found harvesting the explants of English shrub rose 'Heritage' in April to give better regeneration frequency.

The second factor affecting the shoot regeneration was PGR. Every species requires a perfect ratio of PGRs for their physiological development in tissue culture (Armstrong and Johnson 2001). Cytokinins play an important role in *in vitro* shoot organogenesis (Hill 1967), whereas auxins induces rooting in regenerated plants (Martins *et al.* 2013). However, in certain cases auxin act synergistically with cytokinin and support shoot organogenesis (Badere et al. 2002). Thus, it is important to select auxins which promote shoot regeneration without promoting callus formation (George *et al.* 2008) when shoot organogenesis is desired. (Rout *et al.* 1990) suggested that on PGR-free medium, growth rate of *Rosa* sp. is very poor. Hence, cytokinins are required for shoot proliferation. In the present investigation BAP was essential for shoot induction in the explants harvested in rainy and winter seasons. BAP is a purine type of cytokinin which leads to proliferation of axillary bud, which may be due to availability of some common binding sites in plant cells

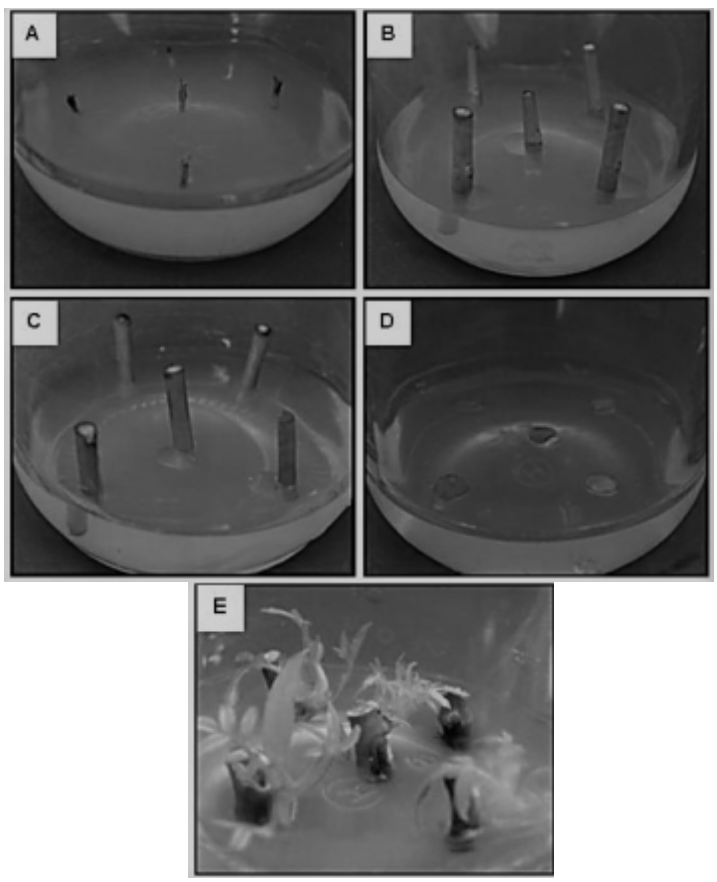

Figure 1: Induction of multiple shoots in *Rosa setigera.* **A-D** Explants inoculated over the nutrient medium. **A.** Shoot tip **B.** Nodal Segment **C.** Internodal Segment **D.** Leaf Disc **E.** Nodal segment with multiple shoots.

(Nielsen *et al.* 1995; George *et al.* 2008). Similar observations have been reported by the other investigators in various species and cultivars of roses (Tabesh *et al.* 2013; Shirdel *et al.* 2013). However, Barna and Wakhlu (1995) and Ibrahim and Debergh (1999) found TDZ to be better than BAP and zeatin in shoot proliferation of *R. hybrida*. Attia *et al.* (2012) found that low concentration of kinetin with BAP is better for shoot proliferation rather than any of the auxin tested in *R. hybrida* cv. Al-Taif. However, Elliot (1970) found that kinetin was incapable to form shoots in rose.

A low concentration of auxin (particularly NAA) with cytokinin was beneficial for shoot induction, although, cytokinin alone was enough. Cytokinins play a major role in plant development, regulation of shoot formation & multiplication and affects orientation in cell-division (Badere *et al*. 2002). Addition of NAA with BAP synergistically increased number of shoots per explant in the present study. These results are in conformation with Taha and Wafa (2012) and Yacoob *et al*. (2014). Similar findings were reported in rose cv. 'Andhra red' (Ganga *et al*. 1998) and *R. hybrida* cv. 'Benjamina paul' (Maurya *et al.* 2013). Khosh-Kui and Sink (1982) reported that highest concentration of NAA besides BAP resulted in callus induction in shoot-tip of *R. hybrida*. George *et al*. (2008) favoured NAA over 2,4-D to induce shoots. Many times 2,4-D with cytokinins promote callus induction. Auxins not only promote root development but are also responsible for cell expansion, cell division and apical dominance. Campanoni and Nick (2005) reported that NAA with low concentration results in cell elongation, while 2,4-D mainly triggers the cell division. *Martin et al.* (2005) suggested that combination of 2,4-D to BAP may induce somatic embryogenesis and suppress the genes involved in organogensis. The other important observation of present investigation is that kinetin was comparatively weaker than BAP in inducing micro-shoots even when auxins were present in the medium. This has also been reported in *Solanum surratense (Pawar et al. 2002)* and *Asparagus maritimus (*Stajner *et al. 2002)*.

CONCLUSION

The present study, thus, proves that the exogenous PGR supplementation influences the regeneration potential of *R. setigera*. This need of exogenous PGRs differs according to the season of harvesting the explant. Hence, to obtain maximum regeneration efficiency we recommend culturing the nodes harvested in rainy season over MS medium containing 2.2µM BAP while those harvested in winter season over MS medium containing 0.5µM NAA and 8.8µM BAP.

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