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Standardization of *ex-vitro* media composition for hardening and acclimatization of *ex- agar* pomegranate plants

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Abstract
In the present study experiments were carried out to standardize *ex-vitro* media composition for hardening, acclimatization and better growth of pomegranate tissue culture *ex-agar* plants developed from nodal explants of var. Bhagwa. The *in-vitro* produced plantlets were washed with running tap water to remove the agar sticking to the roots. The *ex-agar* plantlets were carefully transferred to the pots containing sterilized cocopeat. Inside the shade house, the pro-trays were kept inside the poly tunnels and the poly tunnels are completely closed to avoid entry of air from outside and to maintain required humidity for the establishment of rooted plantlets. After 25 days of the planting in portrays, the plantlets are acclimatized under the shade house and ready for secondary hardening. Thirty primary hardened plantlets were planted in one in each poly bag, which contain potting mixture as per treatment. Ten poly bags were considered as one replication. All these poly bags were kept under the shade house and are watered daily in the morning hours. The survival percentage of plantlets was recorded 30 days after transplanting into the pots. The plants which were survived, established and started to grow were taken into consideration for calculating the percent survival. Among the treatments, highest number of newly formed leaves (21.69) were recorded by cocopeat: vermiculite: vermicompost (1:1:1) (T_4) followed by cocopeat: vermiculite: vermicompost (2:1:1) (T_6) (18.23), while lowest number of newly formed leaves were observed in red soil: vermicompost (1:1) (T_1) (7.43). Among the days, number of newly formed leaves was more at 30th day (19.60) than on 20th day (8.40). Newly formed leaves at 20th day and 30th day recorded that highest number of newly formed leaves (31.33) was recorded by cocopeat: vermiculite: vermicompost (1:1:1) (T_4) on 30th day followed by cocopeat: vermiculite: vermicompost (2:1:1) (T_6) (29.96) on 30th day, whereas, lowest number of newly formed leaves were observed in red soil: vermicompost (1:1) (T_1) (5.10) at 20th day. Among all potting mixture tested, it was observed that maximum survival (83.33 per cent) of plants from nodal explants were found on medium containing red soil: cocopeat: vermicompost (1:2:1).

Keywords: *Ex-vitro* media composition, hardening of tissue culture plants, pomegranate, micropropagation

Introduction
Pomegranate (*Punica Granatum* L.) is an economically important fruit crop of the tropical and subtropical regions of the world. It belongs to the family Punicaceae, which comprises only one genus (Punica) and two species; *P. granatum* and *P. protopunica* (Samir, 2010) [15]. Majorly, it is cultivated for its delicious fruits but the presence of pharmaceutically important tannins and alkaloids in the different plant parts such as the leaves, stem, bark, root and fruit rind further enrich its commercial value (Jayesh and Kumar, 2004) [2]. The fruit juice is a good source of sugars, vitamin C, vitamin B, pantothenic acid, potassium, antioxidant polyphenols, good source of iron, containing polyphenols which inhibit estrogen synthesis and pomegranate seed oil was effective against proliferation of breast cancer cells. The double-flowered pomegranates (which do not bear fruits) are grown in parks and ornamental gardens for their beautiful red flowers (Raj and Kanwar, 2010) [3]. Pomegranates can be grown in variety of soils, ranging from acidic sandy loam to alkaline calcareous soils and even have the adaptability to drought. Because of its ability to grow well in slightly saline soils, it is considered as a saline tolerant plant (Patil and Waghmare, 1983; Rao and Khandelwal, 2001; Arsey *et al*., 2002 Levin, 2006; Marathe *et al*., 2009) [11, 13, 1, 5, 8].

Despite of the fact that India has all the favorable conditions for cultivation of pomegranate, the annual production is very less due to scarcity of good quality disease free planting material of a selected variety. This indicates a need for development of a suitable propagation method for large scale production of the pomegranate plants of a selected variety. Pomegranate is conventionally propagated by hard wood and soft wood cuttings. But, this traditional propagation method has several limitations like low success, very slow propagation, new plants require one year for establishment, not ensure disease-free and healthy plants. In addition, this method is a very time-consuming and labor-intensive process. Hence, there lies an ample scope for large scale multiplication of desired genotypes using micropropagation. Hence, several studies have been conducted on micropropagation of pomegranate trees over the past several years. Protocols have been developed for regeneration of pomegranate plantlets *in vitro* through either organogenesis from callus derived from leaf segments, cotyledons (Murkute *et al*; 2002; Kanwar *et al*., 2010) [7, 3], anthers (Soumendra Naik *et al*., 1999) [16] or through embryogenesis from various seedling explants, petals and immature zygotic embryos (Kanwar *et al*., 2010) [3].

Consequently, this work was designed for *In vitro* multiplication of pomegranate (*P. granatum* L.) 'Bhagwa' cultivar and to study the effect of MS medium and growth regulators for establishment, multiplication and rooting of pomegranate (*P. granatum* L.). Hardening refers to the process of acclimating plants from indoor temperatures to the outdoors. The hardening of *in vitro* raised plantlets is essential for better survival and successful establishment. Transfer of plantlets to soil is the most critical step in micro propagation. The plantlets are maintained under highly protected conditions in *in vitro* i.e. high humidity, low irradiance, low CO2 levels and high sugar content. This is also called acclimatization phase. The high humidity could be generally achieved by covering the plantlets with plastic film under shade together with frequent misting. Shading is necessary since the strong solar light itself may directly damage the plantlets and also the fluctuating solar light intensity with time leads to fluctuation in temperature and relative humidity and hence an excess water loss from the plantlets. As for most horticultural crops, the success of tissue culture is determined by the ability of regenerated plants to be transferred from *in vitro* substrates to soil with as little loss and variability as possible. Successful acclimatization of *in vitro*-regenerated plantlets has been reported in pomegranate using different substrates, albeit with differing rates of success. Naik *et al*., 1999 [9] reported 68% ex vitro survival of cv. „Ganesh" plantlets when transferred to vermicompost which later gave 80% survival upon transfer to soil. Kalalbandi *et al*., 2014 [4] obtained hardening of rooted plantlets ideal with maximum survival of plantlets (71.72%) on medium containing soil + sand (1:1; v/v).

Materials and Methods

In the present investigation total five experiments were carried out in which CRD was applied to find the best treatments for controlling polyphenol exudation, shooting media, rooting media and hardening potting mixtures. The media used for this investigation was Murashige and Skoog's (1962) basal media. The explants were collected from third node of the shoot apex, early in the morning during the spring season in the months of February and March. The type of explant used for the culture was nodal segment with axillary bud, collected during February and March. The explants were collected in the morning, third node from the shoot tip. Controlled polyphenol exudation, shoot and root development were standardized by MS medium with various components. The following nine potting media were used for establishing the *in vitro* produced plantlets after proper sterilization in autoclave at 121 °C.

1. Red soil: Vermicompost (1:1).
2. Cocopeat: Vermicompost (1:1).
3. Vermiculite: Vermicompost (1:1).
4. Cocopeat: Vermiculite: vermicompost (1:1:1).
5. Cocopeat: Vermiculite: vermicompost (1:2:1).
6. Cocopeat: vermiculite: vermicompost (2:1:1).
7. Red soil: cocopeat: vermicompost (1:1:1).
8. Red soil: cocopeat: vermicompost (1:2:1).
9. Red soil: cocopeat: vermicompost (2:1:1).

Uniform *in-vitro* produced plantlets with good number of roots were selected for transplanting in to pro-trays after four weeks of culture period in potting medium.

Cultural Operations

The *in vitro* produced plantlets were washed with running tap water to remove the agar sticking to the roots. The weaned ex agar plantlets were carefully transferred to the pots containing sterilized cocopeat. Inside the shade house, the pro-trays were kept inside the poly tunnels and the poly tunnels are completely closed to avoid entry of air from outside and to maintain required humidity for the establishment of rooted plantlets. After 25 days of the planting in portrays, the plantlets are acclimatized under the shade house and ready for secondary hardening.

Thirty primary hardened plantlets were planted in one in each poly bag, which contain potting mixture as per treatment. Ten poly bags were considered as one replication. All these poly bags were kept under the shade house and are watered daily in the morning hours.

Collection of experimental data

The survival percentage of plantlets was recorded 30 days after transplanting into the pots. The plants which were survived, established and started to grow were taken into consideration for calculating the percent survival.

1. Number of plants survived at 10 days interval (on 10^{th}, 20^{th} and 30^{th} day)
2. Percent of survival of plants on 30^{th} day
3. Length of shoot in cm (at 20th, and 30th day)
4. Number of newly formed leaves (at 20^{th} and 30^{th} day)

Results and Discussion

Number of plants survived at 10 days interval at 10^{th}, 20^{th} and 30^{th} day

The data recorded by using different potting mixtures revealed that the number of plants survived at 10^{th} day of transfer into potting mixture, the maximum number of plants survived (86.66 per cent) was recorded in red soil: cocopeat: vermicompost (1:2:1) (T_7), red soil: cocopeat: vermicompost (1:2:1) (T_8) and red soil: cocopeat: vermicompost (2:1:1) (T_9), followed by cocopeat: vermicompost (1:1) (T_2) and cocopeat: vermiculite: vermicompost (2:1:1) (T_6) i.e., (73.33 per cent) whereas,

lowest number of plants survived (30.00 per cent) was shown by vermiculite: vermicompost (1:1) (T_3) preceded by cocopeat: vermiculite: vermicompost (1:2:1) (T_5) (53.3 per cent) (Table-1).

At 20th day of transfer into potting mixture, the maximum number of plants survived was recorded in red soil: cocopeat: vermicompost (2:1:1) (T_9) and red soil: cocopeat: vermicompost (1:2:1) (T_7) (both 83.33 per cent) followed by red soil: cocopeat: vermicompost (1:2:1) (T_8) (76.66 per cent) whereas, lowest number of plants survived was (30.00 per cent) shown by vermiculite: vermicompost (1:1) (T_3) preceded by cocopeat: vermiculite: vermicompost (1:2:1) (T_5) (53.3 per cent) and cocopeat: vermiculite: vermicompost (2:1:1) (T_6) (50.00 per cent).

At 30th day of transfer into potting mixture, the maximum number of plants survived (80.00 per cent) was recorded in red soil: cocopeat: vermicompost (1:2:1) (T_8) followed by red soil: cocopeat: vermicompost (2:1:1) (T_9) and red soil: cocopeat: vermicompost (1:2:1) (T_7) (both 76.66 per cent); whereas, lowest number of plants survived was (26.66 per cent) recorded by vermiculite: vermicompost (1:1) (T_3) preceded by cocopeat: vermiculite: vermicompost (1:2:1) (T_5) (43.33 per cent).

The treatment, red soil: cocopeat: vermicompost (1:2:1) (T_8) recorded the highest survival of plants (83.33 per cent) followed by red soil: cocopeat: vermicompost (1:2:1) (T_7) (82.20 per cent), whereas, the lowest survival of plants recorded in Red soil: vermicompost (1:1) (T_1) and cocopeat: vermiculite: vermicompost (1:2:1) (T_5) (both 48.88 per cent) preceded by Cocopeat: vermiculite: vermicompost (1:1:1) (54.44 per cent).

Among the days, survival was highest (68.14 per cent) at 10th day followed by survival at 20th day (58.88 per cent), whereas, lowest at 30th day (54.44 per cent)

The interaction among the treatments and days revealed that the survival was highest at 10th day in red soil: cocopeat: vermicompost (1:2:1) (T_7), red soil: cocopeat: vermicompost (1:2:1) (T_8) and red soil: cocopeat: vermicompost (2:1:1) (T_9) (86.66 per cent) followed by red soil: cocopeat: vermicompost (1:2:1) (T_7), red soil: cocopeat: vermicompost (1:2:1) (T_8) on 20th day (both 83.33 per cent), while lowest number of plants survived (26.66 per cent) by vermiculite: vermicompost (1:1) (T_3) on 30th day preceded by vermiculite: vermicompost (1:1) (T_3) at 10th day and vermiculite: vermicompost (1:1) (T_3) (both 30.00 per cent)

The per cent survivality was recorded highest in red soil: cocopeat: vermicompost (1:2:1) (T_8) and red soil: cocopeat: vermicompost (1:1:1) (T_7). Naik *et al*. (1999) [9] reported 68.00 per cent survivality of the plantlets of pomegranate var. Ganesh transferred to vermicompost that lead to 80.00 per cent survival of plants on transferring to soil, plantlets with well-developed roots were successfully acclimatised and eventually established in soil. Plantlets with well-developed roots were successfully acclimatized and eventually established to soil (Naik *et al*. 2000) [10].

Table 1: Number of plants survived at 10 days interval (on 10th, 20th and 30th day) and their interaction

Days	No. of plants survived
10th day	68.14 (56.45)
20th day	58.88 (47.85)
30th day	54.44 (51.65)
CD(P=0.05)	3.10
Treatments	
T_1	48.88 (44.38)
T_2	61.10 (51.58)
T_3	28.88 (32.26)
T_4	54.44 (47.58)
T_5	48.88 (44.30)
T_6	56.66 (49.02)
T_7	82.21 (65.40)
T_8	83.33 (66.14)
T_9	79.99 (64.23))
CD (P = 0.05)	5.51
Days X Treatments interaction	NS

T_1- Red soil:vermicompost (1:1)
T_2- Cocopeat:vermicompost (1:1)
T_3- Vermiculite:vermicompost (1:1)
T_4- Cocopeat: vermiculite: vermicompost (1:1:1)
T_5- Cocopeat: vermiculite: vermicompost (1:2:1)
T_6- Cocopeat: vermiculite: vermicompost (2:1:1)
T_7- Red soil: cocopeat: vermicompost (1:1:1)
T_8- Red soil: cocopeat: vermicompost (1:2:1)
T_9- Red soil: cocopeat: vermicompost (2:1:1)

Per cent survival of plants in 30 days

The per cent of survival of plants in 30 days after transfer into potting mixture shown that highest per cent of survival of plants in 30 days (80.00 per cent) was recorded in red soil: cocopeat: vermicompost (2:1:1) (T_9) followed by both red soil: cocopeat: vermicompost (1:2:1) (T_8) and red soil: cocopeat: vermicompost (1:2:1) (T_7) (76.66 per cent), whereas, lowest per cent of survival of plants in 30 days after transfer into potting mixture was (26.66 per cent) shown by vermiculite: vermicompost (1:1) (T_3) preceded by red soil: vermicompost (1:1) (T_1) (40.00 per cent) (Table-1).

Length of shoot in cm in 20th day and 30th day

At 20th day, the maximum length of shoot (12.50 cm) was recorded in cocopeat: vermiculite: vermicompost (1:1:1) (T_4) followed by cocopeat: vermicompost (1:1) (T_2) (11.66

cm) whereas, lowest length of shoot was (3.73 cm) shown by red soil: vermicompost (1:1) (T_1) preceded by red soil: cocopeat: vermicompost (2:1:1) (T_9) (5.50 cm) (Table-2).
At 30th day, maximum length of shoot (20.33 cm) was recorded in cocopeat: vermicompost (1:1) (T_2) followed by cocopeat: vermiculite: vermicompost (2:1:1) (T_6) (17.10 cm) whereas, lowest length of shoot was (6.43 cm) recorded by red soil: vermicompost (1:1) (T_1) followed by red soil: cocopeat: vermicompost (2:1:1) (T_9) (9.5 cm) (Table-2).
Among the treatments, highest length of shoot (18.45 cm) was observed in cocopeat: vermiculite: vermicompost (1:1:1) (T_4) followed by cocopeat: vermicompost (1:1) (T_2) (15.99 cm), whereas, lowest length of shoot (5.08 cm) observed in red soil: vermicompost (1:1) (T_1) preceded by red soil: cocopeat: vermicompost (2:1:1) (T_9) (7.50 cm). Among the days, on 30th day, highest length of shoot (14.46 cm) was recorded and on 20th day, it was only 7.79 cm only.
The interactions among the treatments and days revealed that the length of shoot was highest on 30th day in cocopeat: vermiculite: vermicompost (1:1:1) (T_4) (24.40 cm) followed by cocopeat: vermicompost (1:1) (T_2) (20.33 cm) on 30th day, while lowest length of shoot (3.73 cm) was recorded in cocopeat: vermicompost (1:1) (T_2) (3.73 cm) on 20th day preceded by cocopeat: vermicompost (1:1) (T_2) (6.43 cm) on 30th day. These are in accordance with Naik *et al*. (1999) reported 68 per cent survivality of the plantlets of pomegranate var. Ganesh transferred to vermicompost that lead 80 per cent survival of plants on transferring to soil, plantlets with well-developed roots were successfully acclimatized and eventually established in soil. Plantlets with well-developed roots were successfully acclimatized and eventually established to soil (Naik *et al*., 2000) [10].

Table 2: Length of shoot in cm (on 20th and 30th day)

S. No.	Treatments	Days		Mean
		20 days	30 days	
1	T_1- Red soil: vermicompost (1:1)	3.73	6.43	5.08
2	T_2-Cocopeat: vermicompost (1:1)	11.66	20.33	15.99
3	T_3-Vermiculite: vermicompost (1:1)	6.60	11.70	9.15
4	T_4-Cocopeat: vermiculite: vermicompost (1:1:1)	12.50	24.40	18.45
5	T_5- Cocopeat: vermiculite: vermicompost (1:2:1)	6.30	13.30	9.80
6	T_6- Cocopeat: vermiculite: vermicompost (2:1:1)	8.80	17.10	12.95
7	T_7- Red soil: cocopeat: vermicompost (1:1:1)	6.43	11.70	9.065
8	T_8- Red soil: cocopeat: vermicompost (1:2:1)	8.60	15.70	12.15
9	T_9- Red soil: cocopeat: vermicompost (2:1:1)	5.50	9.50	7.50
	Mean	7.79	14.46	
	S.Em.±	81	1.18	
	CD (P = 0.05)	2.42	3.52	

Number of newly formed leaves at 20th and 30th day
At 20th day, maximum number of newly formed leaves (12.06) was recorded in cocopeat: vermiculite: vermicompost (1:1:1) (T_4) followed by cocopeat: vermicompost (1:1) (T_2) (10.16) whereas, lowest number of newly formed leaves (5.10) was recorded by red soil: vermicompost (1:1) (T_1) preceded by red soil: cocopeat: vermicompost (2:1:1) (T_9) (6.20) (Table-3).
At 30th day, maximum number of newly formed leaves (31.33) was recorded in cocopeat: vermiculite: vermicompost (1:1:1) (T_4) followed by cocopeat: vermiculite: vermicompost (2:1:1) (T_6) (26.96) whereas, lowest number of newly formed leaves (9.76) was recorded by red soil: vermicompost (1:1) (T_1) preceded by red soil: cocopeat: vermicompost (2:1:1) (T_9) (14.03) (Table-3).
Among the treatments, highest number of newly formed leaves (21.69) were recorded by cocopeat: vermiculite: vermicompost (1:1:1) (T_4) followed by cocopeat: vermiculite: vermicompost (2:1:1) (T_6) (18.23), while lowest number of newly formed leaves were observed in red soil: vermicompost (1:1) (T_1) (7.43) preceded by red soil: cocopeat: vermicompost (2:1:1) (T_9) (10.11). Among the days, number of newly formed leaves was more at 30th day (19.60) than on 20th day (8.40).
The interaction effect between different treatments and days on number of newly formed leaves at 20th day and 30th day recorded that highest number of newly formed leaves (31.33) was recorded by cocopeat: vermiculite: vermicompost (1:1:1) (T_4) on 30th day followed by cocopeat: vermiculite: vermicompost (2:1:1) (T_6) (29.96) on 30th day, whereas, lowest number of newly formed leaves were observed in red soil: vermicompost (1:1) (T_1) (5.10) at 20th day preceded by red soil: vermicompost (1:1) (T_1) (9.76) at 30th day and cocopeat: vermicompost (1:1) (T_2) (10.16) at 20th day (Table-4).
Among all potting mixture tested, it was observed that maximum survival (83.33 per cent) of plants from nodal explants were found on medium containing red soil: cocopeat: vermicompost (1:2:1) followed by red soil: cocopeat: vermicompost (1:1:1) (82.21 per cent) among the potting mixtures tested (Table-1) (Fig-3).

Table 3: Number of newly formed leaves (at 20th and 30th day)

S. No.	Treatments	Days		Mean
		20 days	30 days	
1	T_1- Red soil: vermicompost (1:1)	5.10 (2.36)	9.76 (3.19)	7.43 (2.77)
2	T_2-Cocopeat: vermicompost (1:1)	10.16 (3.26)	18.20 (4.31)	14.18 (3.78)
3	T_3-Vermiculite: vermicompost (1:1)	7.16 (2.76)	18.33 (4.32)	12.74 (3.54)
4	T_4-Cocopeat: vermiculite: vermicompost (1:1:1)	12.06 (3.53)	31.33 (5.58)	21.69 (4.55)
5	T_5- Cocopeat: vermiculite: vermicompost (1:2:1)	8.13 (2.91)	21.16 (4.65)	14.64 (3.78)

6	T_6- Cocopeat: vermiculite: vermicompost (2:1:1)	9.50 (3.15)	26.96 (5.23)	18.23 (4.19)
7	T_7- Red soil: cocopeat: vermicompost (1:1:1)	7.93 (2.87)	17.50 (4.21)	12.71 (3.54)
8	T_8- Red soil: cocopeat: vermicompost (1:2:1)	9.43 (3.13)	19.16 (4.40)	14.29 (3.76)
9	T_9- Red soil: cocopeat: vermicompost (2:1:1)	6.20 (2.58)	14.03 (3.80)	10.11 (3.19)
	Mean	8.40 (2.95)	19.60 (4.41)	
	S.Em.±	0.18	0.27	
	CD (P = 0.05)	0.54	0.81	

Table 4: Number of newly formed leaves (at 20th and 30th day) and their interaction

Days	**No. of newly formed leaves**
20th day	8.40 (2.95)
30th day	19.60 (4.41)
CD (P = 0.05)	0.32
Treatments	
T_1	7.43 (2.77)
T_2	14.18 (3.78)
T_3	12.74 (3.54)
T_4	21.69 (4.55)
T_5	14.64 (3.78)
T_6	18.23 (4.19)
T_7	12.71 (3.54)
T_8	14.29 (3.76)
T_9	10.11 (3.19)
CD(P=0.05)	0.46
Days X Treatments interaction	NS

T_1- Red soil:vermicompost (1:1)
T_2- Cocopeat:vermicompost (1:1)
T_3- Vermiculite:vermicompost (1:1)
T_4- Cocopeat: vermiculite: vermicompost (1:1:1)
T_5- Cocopeat: vermiculite: vermicompost (1:2:1)
T_6- Cocopeat: vermiculite: vermicompost (2:1:1)
T_7- Red soil: cocopeat: vermicompost (1:1:1)
T_8- Red soil: cocopeat: vermicompost (1:2:1)
T_9- Red soil: cocopeat: vermicompost (2:1:1)

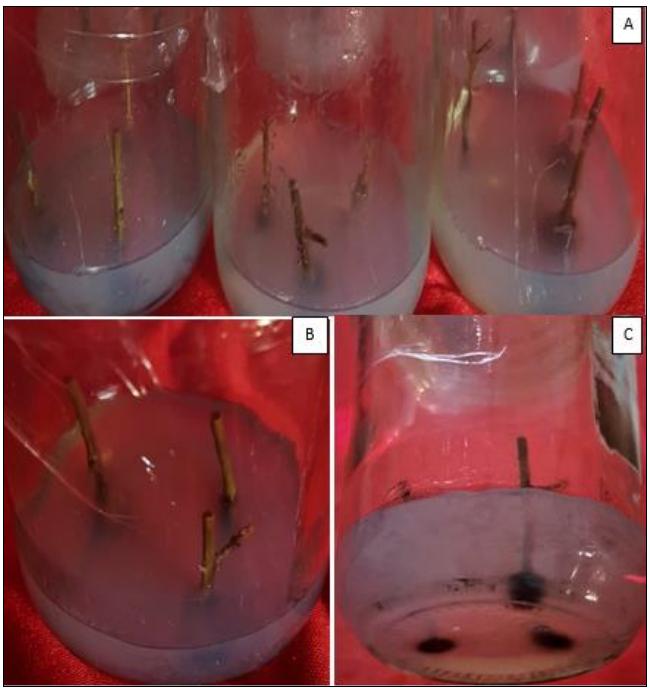

Fig 1: a) Inoculation of explants in culture bottles, b) Explants in the media immediately after inoculation, c) Explants in the media two days after inoculation

Fig 2: a) Fungal contamination after two days of inoculation of explant, b) Fungal contamination after four days of inoculation of explant, c) Bacterial contamination after inoculation of explant

Fig 3: Primary hardened rooted plantlets of pomegranate cv. Bhagwa in sterilized cocopeat

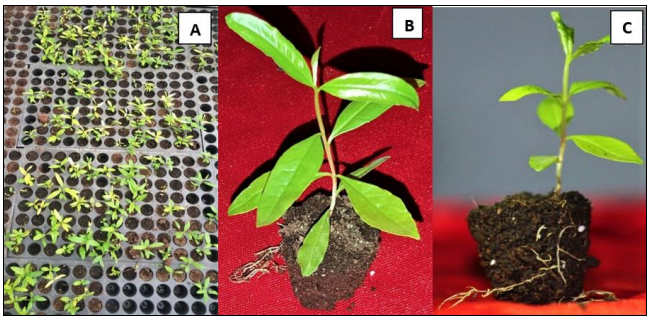

Fig 4: Secondary hardened plantlets of pomegranate cv. Bhagwa in, a) Red soil: cocopeat: vermicompost (2:1:1) (T_8), b) Red soil: cocopeat: vermicompost (1:2:1) (T_8), c) Fully hardened pomegranate plants ready for plating

Fig 5: Secondary hardened plantlets of pomegranate cv. Bhagwa in, a) Red soil: cocopeat: vermicompost (2:1:1) (T_8), b) Red soil: cocopeat: vermicompost (1:2:1) (T_8), c) Fully hardened pomegranate plants ready for plating

Conclusion

In the present investigation different *ex-vitro* media compositions for hardening, acclimatization and better growth of pomegranate tissue culture *ex-agar* plants were tested. Among the treatments, highest number of newly formed leaves (21.69) were recorded by cocopeat: vermiculite: vermicompost (1:1:1) (T_4) followed by cocopeat: vermiculite: vermicompost (2:1:1) (T_6) (18.23). Among the days, number of newly formed leaves was more at 30th day (19.60) than on 20th day (8.40). Newly formed leaves at 20th day and 30th day recorded that highest number of newly formed leaves (31.33) was recorded by cocopeat: vermiculite: vermicompost (1:1:1) (T_4) on 30th day followed by cocopeat: vermiculite: vermicompost (2:1:1) (T_6) (29.96) on 30th day, whereas, lowest number of newly formed leaves were observed in red soil: vermicompost (1:1) (T_1) (5.10) at 20th day preceded by red soil: vermicompost (1:1) (T_1) (9.76) at 30th day and cocopeat: vermicompost (1:1) (T_2) (10.16) at 20th day. Considering the survival per cent of primary hardened plants, shoot length and newly formed leaves, it was concluded that red soil: cocopeat: vermicompost (1:2:1) was the best followed by red soil: cocopeat: vermicompost (1:1:1) among the potting mixtures tested.

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