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Laxmi Bagewadi
Department of Fruit Science, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

RV Tank
Department of Fruit Science, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

SL Chawla
Department of Floriculture and Landscape Architecture, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

Kitty Rajan
Department of Floriculture and Landscape Architecture, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

Correspondence
Laxmi Bagewadi
Department of Fruit Science, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari, Gujarat, India

Response of chemicals and colour shade nets on growth of epicotyl grafting of mango (*Mangifera indica* L.) CV. Kesar

Laxmi Bagewadi, RV Tank, SL Chawla and Kitty Rajan

Abstract
An experiment was conducted to study the effect of chemicals and colour shade nets on growth of epicotyl grafting of mango (*Mangifera indica* L.) cv. Kesar at Regional Horticultural Research Station, ASPEE College of Horticulture & Forestry, Navsari Agricultural University, Navsari during the year 2017-18. The experiment was laid out in completely randomized design with two factors repeated three times. The treatment comprised of 3 different chemicals as scion dip *viz*., No application of chemical (C_1), BAP @ 20 mgl^{-1} for 5 second (C_2) and BAP (20 mgl^{-1}) +$ZnSO_4$ (750 mgl^{-1}) (C_3) for 5 second and five different colour shade nets *viz*., open field condition (P_1), green colour shade net (P_2), red colour shade net (P_3), white colour shade net (P_4) and blue colour shade net (P_5). Results of present investigation revealed that the scion treated with BAP 20 mgl^{-1} + $ZnSO_4$ 750 mgl^{-1} for 5 seconds (C_3) was found better for early sprouting, graft sprouting percentage, number of graft success and survival percentage, number of leaves, total leaf area, root length and number of roots and fresh and dry weight of roots. Whereas, BAP @ 20 mgl^{-1} for 5 seconds (C_2) was found beneficial for incremental height of graft, incremental girth of scion and fresh and dry weight of shoot.
Among the different colour shade nets, mango epicotyl grafts kept under green colour shade net (P_2) was found effective with respect to graft success parameters (days taken for sprouting, graft sprouting percentage, number of graft success and survival percentage and shoot parameters *viz*., incremental height of graft, number of leaves, total leaf area, fresh and dry weight of shoot. Similarly, root parameters (number of roots, root length, fresh and dry weight of roots) were also found superior in green colour shade net. While, girth of scion was found maximum under red and compared to other colour shade nets.
Considering the interaction effect between chemicals and colour shade nets, maximum fresh and dry weight of shoot and root length were found when scion treated with BAP 20 mgl^{-1} for 5 seconds and grafts kept under green colour shade net (C_2P_2) at 6 months after grafting. While, maximum number of leaves and total leaf area were recorded with the C_3P_2 and C_1P_2 treatment combinations, respectively at 6 months after grafting.

Keywords: Sprouting, BAP, $ZnSO_4$, mango, colour shade net and chemicals

Introduction
Mango (*Mangifera indica* L.) is the national fruit of India. Mango is a native to South East Asia, especially Burma and Eastern India. India shares about 39% of total mango production in the world. Area under mango in India is about 2263.48 thousand ha and production 19,687 thousand MT with the productivity of 7.32 MT / ha, while in Gujarat area under mango is about 161.2 thousand ha and production 1424.8 thousand MT (Anon., 2017). Kesar is the leading commercial variety of Gujarat and have great export potential owing to its attractive skin colour, shape, size, pleasant aroma, rich flavor and excellent taste and good keeping quality.
In Gujarat, BAIF, Dhruva adopted epicotyl grafting method for mango propagation in large scale. Recent studies correlate the increasing influence of the plant growth regulators and their complex interaction to plant system having significant effect on their growth and development for successful formation of graft union. Plant growth regulator like BAP and chemical $ZnSO_4$, when applied to graft union at the time of grafting as well as pre- treatment of root stock and scion resulted in earlier callus formation and graft union (Ikeuchi *et al*., 2013) [7]. Higher plants respond differently to different light quantity, quality, direction and periodicity (Stamps, 2009) [18] and have been possible to manipulate plant morphology and physiology using colour shade nets (Rajapkse *et al*., 1999 and Shahak, 2008) [15, 17].

Materials and Methods

The present investigation was carried out during 2017-18 at Regional Horticultural Research Station, ASPEE College of Horticulture and Forestry, Navsari Agricultural University, Navsari. The treatment comprised of 3 different chemicals as scion dip *viz*., No application of chemical (C_1), BAP @ 20 mgl^{-1} for 5 second (C_2) and BAP (20 mgl^{-1}) + $ZnSO_4$ (750 mgl^{-1}) (C_3) for 5 second and five different colour shade nets *viz*., open field condition (P_1), green colour shade net (P_2), red colour shade net (P_3), white colour shade net (P_4) and blue colour shade net (P_5).

The experiment was laid out in Completely Randomized Design with factorial concept (FCRD). Twenty epicotyl grafts were made in each treatment. Regarding methodology, stones were collected from processing factory. They were sown in line on the flat beds in flat position at 10 cm x 2.5 cm distance and were covered with 5 cm thick layer of farm yard manure and soil in the ratio of 1:1. The healthy vigorous and strong seedlings of 15 days old with straight and stout epicotyl having coppery leaves were uprooted along with stone for making epicotyl grafts. The healthy mature terminal shoots of more than 3 month age with plumpy buds were selected from healthy mother trees. The epicotyl grafting was done by wedge technique of grafting as described by Bhan *et al*. (1969) [4].

Before grafting cut portion of scion was dipped in beaker for 5 second containing freshly prepared solution. Epicotyl grafts were placed in 9" × 7" in size polybag having drainage holes at the lower end and filled with media. All the grafted plants in polythene bags were transferred to respective colour shade nets as per treatment. The data on days taken for sprouting, sprouting percentage, graft success, incremental height and girth of grafts, number of leaves per graft at 2 month interval. Whereas other growth parameters such as total leaf area/ graft, root length, no. of roots, fresh weight of shoot and root, dry weight of shoot and root and survival percentage at 6 months after grafting. The data recorded during the course of investigation were subjected to statistical analysis following standard procedure described by Panse and Sukhatme (1967).

Results and Discussion

Graft - success characters

Days taken for first sprouting

Regarding the effect of chemicals, the minimum number of days taken for first sprouting (9.10) was observed in the scion treated with BAP (20 mgl^{-1}) + $ZnSO_4$ (750 mgl^{-1}) (C_3). This might be due to the abundant supply of carbohydrates, other food material and defoliation which initiates bud activation which is based on the principle that when photosynthetic unit of shoots are removed a sink created in defoliated shoots and the reserve food material from the adjoining shoots get mobilized to the defoliated shoots and they are in position to sprout early (Zimmerman, 1958) [20].

Among colour shade nets, the minimum number of days taken for first sprouting (8.33) was observed under green colour shade net (P_2), which was at par with red colour shade net (8.51) and blue colour shade net (8.92). There may be some agro-climatic factors responsible for the delayed sprouting and high temperature may also be the limiting factor for the sprouting of mango epicotyl grafts (Mahajan *et al*., 2017) [14].

Graft sprouting (%) at 1 month

The maximum graft sprouting percentage (60.34) was observed significantly with the application of BAP 20 mgl^{-1} + $ZnSO_4$ 750 mgl^{-1} (C_3) at one month after grafting (C_1). Also it might be due to abundant supply of carbohydrates, other food material and defoliation which initiates bud activation which is based on the principle that when photosynthetic unit of shoots are removed a sink created in the defoliated shoots and the reverse food material from the adjoining shoots get mobilized to the defoliate shoots and they are in position to sprout early (Zimmerman, 1958) [20]. Regarding colour shade nets, Maximum graft sprouting percentage (67.11) was noticed under green colour shade net (P_2) at one month after grafting.

Graft success

The maximum number of successful grafts were obtained significantly in epicotyl grafts (14.02, 12.82 and 11.64) when treated with BAP 20 mgl^{-1} + $ZnSO_4$ 750 mgl^{-1} (C_3) at 2, 4 and 6 months after grafting respectively. Zinc help in maintenance of IAA which lead to higher accumulation of IAA, thereby leading to the formation of xylem in graft union after phloem formation (Masev and Kutacek, 1966) [9].

Among colour shade nets higher number of successful grafts were (16.91, 15.38 and 14.62) noted significantly in epicotyl grafts under green colour shade net (P_2) at 2, 4 and 6 months after grafting respectively. This influenced might be due to various factors like variation in the light quality, increased photosynthesis, moderate temperature and relative humidity (Hasanein *et al*., 2011) [21] result was in conformity with the observation made by Anushma *et al*. (2014) [2] in Jamun.

Survival percentage at 6 month

Significantly the maximum survival percentage of epicotyl grafts (49.94) was obtained when scion treated with BAP 20 mgl^{-1} + $ZnSO_4$ 750 mgl^{-1} (C_3) at 6 months after grafting. Cytokinin is the major developmental signal of the root, is involved in the differentiation of fibres, vessels and sieve tubes. It gets accentuated in the presence of auxin, stimulates the early stages of vascular differentiation, at the time when many cells divisions occur in the differentiating tissue. Regarding colour shade nets, the maximum survival percentage of grafts (58.79) was observed significantly in epicotyl grafts kept under green colour shade net (P_2) which was at par with red colour shade net (P_3). The results of study are in close agreement with the findings of Isikalan *et al*. (2011) [8] in almond.

Incremental height of graft (cm)

The maximum height of graft incremented significantly at 2, 4 and 6 months after grafting i.e. 2.76, 5.39 and 8.24 cm, respectively, was recorded when the scion was treated with BAP 20 mgl^{-1} which was at par C_3 treatment. It may be due to accumulation of greater photosynthesis leading to better growth parameters and also due to the role of cytokinin in increasing cell division in apical meristems and cambium (Mazher *et al*. (2011) [10].

Regarding colour shade nets, results revealed higher incremental height of graft *viz*. 3.16, 6.12 and 9.22 cm was recorded in grafts kept under green colour shade net (P_2) at 2, 4 and 6 months after grafting. It is due to the fact that blue nets do not transmit light between 580 and 750 nm, thus keeping the R/FR ratio similar to that of natural sunlight. Thus the lack of FR (700-750 nm) may be major inducer of dwarfing and inhibition effects in blue nets (Oren-Shamir *et al*., 2001) [11].

Incremental girth of scion (mm)

The maximum incremented girth of scion *viz*., 0.317, 0.433

and 0.667 mm was recorded significantly when the scion was treated with BAP 20 mgl^{-1} (C_2) at 2, 4 and 6 months after grafting respectively at 6 months after grafting. With respect to colour shade nets, the maximum incremented girth of scion (0.371, 0.490 and 0.760 mm) was measured significantly under red colour shade net (P_3) at 2, 4 and 6 months after grafting, respectively.). In shade nets, lower temperature because of 50 % entry of sunlight; higher humidity, lower leaf temperature and lower PAR might have increased stomatal conductance leading to higher assimilation of photosynthates in plants. The night temperature might have influenced on respiration process positively for releasing energy required for plant growth and the ability of green coloured shade net to scatter more light gives more diffused radiation resulting into increased absorbed photo synthetically active radiation (Baryalari and Kulkarni, 2015) [22].

The number of leaves

Maximum number of leaves (13.03, 16.54 and 23.27) at 2, 4 and 6 months after grafting, respectively were found in epicotyl grafts when scion was dipped in BAP (20 mgl^{-1}) + $ZnSO_4$ (750 mgl^{-1}) treatment (C_3) at 2, 4 and 6 month after grafting respectively. It was due to accumulation of greater photosynthates leading to better growth parameters (Mazher *et al*., 2011) [10].

Regarding colour shade nets maximum number of leaves (14.61, 18.23 and 26.30 leaves) at 2, 4 and 6 months after grafting, respectively were found under green colour shade net (P_2). It may be due to the favourable condition like lower air and leaf temperature, higher humidity use full to maintain turgidity in all through reduction in evapotranspiration rate, higher stomatal conductance and thereby higher photosynthesis might have produced good amount of raw food material to induce higher number of leaves and leaf area (Desai *et al*. 2015) [5].

Total leaf area

The higher total leaf area (898.88 cm^2) was noted significantly with dipping of scion cut end with the treatment of BAP 20 mgl^{-1} + $ZnSO_4$ 750 mgl^{-1} for 5 seconds (C_3) at 6 months after grafting. Zinc is constituent of other enzymes involved in photosynthesis, including ribulose 1, 5-biphosphate carboxylase (RuBPC) which has been found to catalyse the initial step of carbon dioxide fixation. It also acts to increase chlorophyll content and structure (Brown *et al*., 1993) [23]. This might lead to Increased in the unit leaf area of grafts.

Regarding to colour shade nets, maximum leaf area of graft (986.52 cm^2) was noted under green colour shade net (P_2) at 6 months after grafting at 6 months after grafting. It might be due to the favourable effect of shade and increased photosynthetic area through the action in cell division and cell enlargement (Pandey and Sinha, 2004) [12].

Root length (cm)

The scion treated with BAP (20 mgl^{-1}) + $ZnSO_4$ (750 mgl^{-1}) (C_3) had showed a maximum root length (32.40 cm) at 6 months after grafting. This improvement in root parameters due to BAP and $ZnSO_4$ chemicals resulted that callus formation at graft union with BAP positively influenced the root formation and there is a correlation between root initiation and auxin movement (Hartman *et al*., 1990). With respect to colour shade nets, the maximum root length (35.13 cm) was measured under green colour shade net (P_2) at 6 month after grafting. This is in line with the results of Baiyeri, 2006 [3] in papaya.

No. of roots

The scion treatment consisting of BAP (20 mgl^{-1}) + $ZnSO_4$ (750 mgl^{-1}) *i.e.* (C_3) resulted in maximum number of roots (8.88) at 6 months after grafting which was significantly higher over all other chemical treatments. Under all the shade nets, root length was increased, but it was higher under green colour shade net due to developed root system could have inspired better uptake resulting the higher growth. Maximum fresh weight of root was observed under green colour shade net as compared to the open field condition. This is in line with the results of Baiyeri, 2006 [3] in papaya.

Regarding colour shade nets, the maximum number of roots (9.67) were observed under green colour shade net (P_2) at 6 months after grafting. The element zinc is required for synthesis of tryptophan which is precursor of IAA, it also has an active role in the production of an essential growth hormone auxin (Hafeez, 2013) [6].

Fresh weight of shoot (g)

The scion treated with BAP 20 mgl^{-1} (C_2) exhibited significantly the maximum fresh weight of shoot (22.76 g) at 6 months after grafting. Regarding colour shade nets, maximum fresh weight of shoot (25.90 g) was recorded under green colour shade net (P_2) at 6 months after grafting. The results were in conformity with the observation made by Patil *et al*. (2010) [13] in rose.

Fresh weight of roots (g)

The maximum fresh weight of roots (9.49 g) was noticed in scion treatment consisting of BAP (20 mgl^{-1}) + $ZnSO_4$ (750 mgl^{-1}) *i.e.* (C_3). Regarding colour shade nets, significantly the maximum fresh weight of roots (11.09 g) was recorded under green colour shade net (P_2) at 6 months after grafting.

Table 1: Effect of chemicals and colour shade nets on days taken for sprouting and sprouting (%) at one month, graft success, and survival % in epicotyl grafting of mango.

Treatments	Days taken for sprouting	Graft sprouting (%) at 1 month	Survival (%)	Incremental height of graft(cm)		
			6 MAG	2 MAG	4 MAG	6 MAG
Chemicals (C)						
C_1-No application	9.78	56.13 (68.18)	45.41 (50.71)	2.50	4.86	7.45
C_2-BAP (20 mgl^{-1})	9.44	57.97 (70.83)	47.66 (54.45)	2.76	5.39	8.24
C_3-BAP (20 mgl^{-1}) + $ZnSO_4$ (750 mgl^{-1})	9.10	60.34 (74.09)	49.94 (58.19)	2.63	5.12	7.83
S.Em. ±	0.17	0.97	0.84	0.06	0.11	0.18
C.D. at 5%	0.48	2.79	2.44	0.16	0.31	0.53
Colour shade nets (P)						
P_1- Red colour shade nets	11.07	45.24 (50.45)	37.11 (36.56)	2.34	4.57	7.15
P_2- Red colour shade nets	8.33	67.11 (84.66)	58.79 (73.08)	3.16	6.12	9.22

P_3 –Red colour shade nets	8.51	65.02 (81.90)	55.76 (68.25)	2.97	5.76	8.53
P_4 - Red colour shade nets	10.38	49.74 (58.24)	40.36 (42.01)	2.61	5.09	7.84
P_5- Red colour shade nets	8.92	63.61 (79.90)	46.34 (52.35)	2.07	4.06	6.46
S.Em. ±	0.21	1.25	1.09	0.07	0.14	0.24
C.D. at 5%	0.62	3.60	3.15	0.21	0.39	0.69
Interaction (C X P)						
S.Em. ±	0.37	2.16	1.89	0.13	0.24	0.41
C.D. at 5%	NS	NS	NS	NS	NS	NS
C.V.%	6.79	6.44	6.8	8.43	7.99	9.12

Table 2: Effect of chemicals and colour shade nets on Incremental girth of scion, number of leaves, total leaf area, root length, no. of roots, fresh weight of shoot, Fresh weight of root, dry weight of shoot and dry weight of root in epicotyl grafting of mango.

Treatments	Incremental girth of scion (mm)			number of leaves			total leaf area (cm^2)	Root length (cm)	No. of roots	Fresh weight of shoot (g)	Fresh weight of roots (g)	Dry weight of shoot (g)	Dry weight of roots (g)
	2 Mag	4 Mag	6 Mag	2 Mag	4 Mag	6 Mag							
Chemicals (C)													
C_1 - No application	0.257	0.383	0.601	11.27	14.41	20.41	821.43	28.49	7.92	21.14	8.68	10.11	3.32
C_2 - BAP (20 mgl^{-1})	0.317	0.433	0.667	12.23	15.52	21.86	860.24	31.78	8.43	22.76	9.11	11.16	3.53
C_3 - BAP (20 mgl^{-1}) + $ZnSO_4$ (750 mgl^{-1})	0.304	0.418	0.656	13.03	16.54	23.27	898.88	32.40	8.88	21.78	9.49	10.58	3.74
S.Em. ±	0.004	0.006	0.007	0.28	0.37	0.49	18.78	0.55	0.11	0.39	0.19	0.21	0.08
C.D. at 5%	0.012	0.016	0.021	0.82	1.06	1.42	54.24	1.59	0.31	1.13	0.54	0.61	0.23
Colour shade nets (P)													
P_1 - Open field condition	0.210	0.320	0.481	9.67	12.78	17.24	743.90	26.58	7.04	18.44	7.54	8.43	3.19
P_2 - Green colour shade net	0.349	0.449	0.731	14.61	18.23	26.23	986.52	35.13	9.67	25.90	11.09	12.90	4.34
P_3 - Red colour shade net	0.371	0.490	0.760	13.62	16.89	24.51	829.04	33.06	9.29	24.54	10.49	12.16	4.14
P_4 - White colour shade net	0.239	0.380	0.562	10.88	14.11	19.47	794.60	30.67	8.34	19.34	8.74	9.19	3.25
P_5 - Blue colour shade net	0.294	0.419	0.672	12.10	15.46	21.71	946.85	29.00	7.71	21.23	7.62	10.41	2.73
S.Em. ±	0.006	0.007	0.009	0.37	0.47	0.63	24.25	0.71	0.14	0.50	0.24	0.27	0.10
C.D. at 5%	0.016	0.021	0.027	1.06	1.36	1.83	70.02	2.05	0.40	1.45	0.70	0.79	0.30
Interaction (C X P)													
S.Em. ±	0.010	0.012	0.016	0.64	0.82	1.10	42.00	1.23	0.24	0.87	0.42	0.47	0.18
C.D. at 5%	NS	NS	NS	NS	NS	3.17	121.28	3.55	NS	2.52	NS	1.36	NS
C.V.%	5.67	5.19	4.33	9.04	9.15	8.71	8.46	6.90	4.97	6.90	8.00	7.70	8.72

MAG- Month after grafting

Dry weight of shoot (g)

The scion treatment consisting of BAP (20 mgl^{-1}) *i.e.* (C_2) resulted in maximum dry weight of shoot (11.16 g) at 6 months after grafting. Regarding colour shade nets, significantly maximum dry weight of shoot (12.90 g) was observed under green colour shade net (P_2). The results were in conformity with the observation made by Yamashita *et al*. (2006) [19] in mango.

Dry weight of roots (g)

Significantly maximum dry weight of roots (3.74 g) was noted when the scion was treated with BAP (20 mgl^{-1}) + $ZnSO_4$ (750 mgl^{-1}) *i.e.* (C_3) at 6 months after grafting. Among colour shade nets, significantly maximum dry weight of roots (4.34 g) was recorded under green colour shade net (P_2), which was at par with red colour shade net (P_3) at 6 months after grafting.

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