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Standardization of suitable month and growing conditions for softwood grafting in acid lime (*Citrus aurantifolia* L.) cv. Kagzi on Rangpur lime rootstock

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Abstract

An investigation on Acid lime (*Citrus aurantifolia* L.) cv. Kagzi is an important citrus crop valued for its high acidity, juice content and year-round demand, but seed propagation causes genetic variability and a long juvenile phase, making vegetative propagation necessary. Softwood grafting on Rangpur lime (*Citrus limonia* Osbeck) was standardized during 2024 -25 at KRCCCH, Arabhavi, to identify the best grafting month and growing environmental conditions. Results showed significant effects of month, growing conditions and their interactions on sprouting, growth, vigour, biomass and survival, with polyhouse showing earliest sprouting (20.85 days) and superior growth, and 15th January recording maximum vigour and biomass. The combination C3M6 (15th January under poly house) recorded highest graft success (90.74%) and survival (92.86%). Thus, softwood grafting during mid-January under polyhouse conditions is recommended for producing vigorous, true-to-type, high-quality Kagzi lime plants.

Keywords: Acid lime, Kagzi, Rangpur lime, softwood grafting, graft success, survival percentage

1. Introduction

Citrus (*Citrus spp.*) belonging to the family Rutaceae represents one of the most important subtropical and tropical fruit groups, valued for its nutritional, therapeutic and economic significance (Rajput and Sri, 1999) ^[14]. Commonly known as "Patilebu" in Bengali, "Kaghzinimbu" in Hindi, "Nimbuk" in Sanskrit, "Nimma" in Tamil and "Limbe" in Kannada, citrus fruits are widely consumed globally (Karmakar, 2013) ^[9]. India is a major citrus-producing country, ranking third in the world, with cultivation over 1.09 million ha and a production of 14.7 million tonnes (Anon., 2024) ^[2]. Within India, acid lime (*Citrus aurantifolia*) holds significant commercial value due to its high titratable acidity, elevated ascorbic acid content and extensive use in the fresh market and processing industries.

To maintain clonal fidelity and orchard uniformity, acid lime requires vegetative multiplication. The cultivar Kagzi lime is predominantly used as a scion owing to its superior physico-chemical fruit attributes, such as thin pericarp, high juice recovery and strong consumer preference (Shivankar, 2011) ^[16]. Successful graft establishment, however, is greatly influenced by the rootstock. Rangpur lime (*Citrus limonia*) is widely used as a rootstock due to its polyembryonic seed nature (ensuring true-to-type progeny), high graft compatibility, vigorous growth, drought tolerance and resistance to certain biotic stresses, thereby enhancing scion vigor, productivity and orchard longevity (Singh *et al.*, 2012) ^[17].

Softwood grafting is one of the most efficient clonal propagation techniques in citrus, but the success rate is significantly affected by environmental conditions, particularly months and environmental growing conditions. Therefore, standardizing the optimum grafting time and ideal growing conditions is essential for producing quality planting material of Kagzi lime on Rangpur lime rootstock.

2. Material and Methods

The experiment was carried out during 2024-25 at the Department of Fruit Science, Kittur Rani Channamma College of Horticulture, Arabhavi, Karnataka.

The experimental site is located at 16°15' North latitude, 74°45' East longitude. Arabhavi lies in Zone-3, Region- 2 of agro climatic zones of Karnataka. The treatments comprised of three levels of growing conditions (C) viz., C1- Open condition, C2- Shade house, C3- Poly house and Six levels of different Grafting months (M) viz., M1 - August (2024), M2 - September (2024), M3 -October (2024), M4 - November (2024), M5 - December (2024), M-6 - January (2025). The experiment was laid out in Factorial Completely Randomized Design (FCRD) with Factorial concept comprising eighteen treatment combinations with three replications. Total 540 healthy Rangpur lime compatible rootstocks were used for this experiment i.e., 30 plants per treatment.

2.1 Collection of fruits and extraction of seeds

Graft union success in citrus depends on the rootstock, as it regulates root growth and nutrient transfer to the scion. Mature fruits of Rangpur lime were collected from a mother tree at KRCCH, Arabhavi, and the extracted seeds were sown to produce uniform polyembryonic seedlings. Vigorous six to eight-month-old Rangpur lime seedlings grown in polybags (15 × 25 cm) containing soil: sand: FYM (2:1:1) and measuring 30 - 40 cm in height with 0.8 - 1.0 cm stem diameter were selected for grafting.

2.2 Selection of scion and collection

A vigorous, consistently bearing mother plant, 9-10 years old and known for high yield and superior fruit quality, was selected from the Department of Fruit Science, KRCCH, Arabhavi, for obtaining scion material. Well-developed current-season shoots served as scion wood. These shoots were defoliated with a secateur by retaining only the petiole stubs, which stimulates bud swelling; this was performed 8-10 days prior to grafting. On the grafting day, scion sticks of 10-15 cm length were collected from the current flush early in the morning (08:00 - 09:00 AM). Immediately after harvesting, the scions were packed in moist cloth and enclosed in a polythene bag to avoid moisture loss. To ensure protection against fungal pathogens, the scions were subsequently treated by dipping them in a 0.1 per cent carbendazim solution.

2.3 Method of grafting

For softwood grafting, vigorous and pathogen-free rootstocks were beheaded at 15-20 cm above the substrate using a sterilized grafting knife. Scion twigs were carefully selected to ensure uniform girth with the stock. A longitudinal cleft incision of approximately 4-5 cm was made on the decapitated stem to produce a V-shaped split. The basal portion of the scion was fashioned into a tapered wedge of about 5 cm in length and inserted firmly into the V-shaped cleft of the rootstock. The graft union was secured with a tightly wrapped polythene strip, and each graft was enclosed with a small polythene cap to minimize moisture loss and maintain a humid micro-environment. Routine irrigation and manual weed removal were undertaken in the

polybags.

For data recording, five grafts per treatment were utilized. Parameters including days to initial sprouting, number of sprouts per graft, sprout length, leaf number, graft height and graft vigour index were recorded at 120 days after grafting. Graft success percentage was assessed at 90 days, while survival percentages were evaluated at 120 days after grafting. Statistical interpretation of the recorded traits was carried out using analysis of variance (ANOVA) under a Completely Randomized Design (CRD) with factorial concept, following the methodology of Panse and Sukhatme (1985) ^[12]. Graft vigour index, graft success and survival percentages were computed using standard formulae.

$$\text{Graft Success (\%)} = \frac{\text{Number of grafts successful at the end of the experiment}}{\text{Number of grafts used for treatment}} \times 100$$

$$\text{Graft Survival (\%)} = \frac{\text{Number of grafts that remained alive at the end of the experiment}}{\text{Number of successful grafts}} \times 100$$

3. Results and Discussion

3.1 Days taken for sprouting parameters

Sprouting time of acid lime cv. Kagzi grafted onto Rangpur lime varied significantly across growing environments and grafting months. Grafts grown under poly house conditions exhibited the earliest sprouting (20.85, 38.85 and 65.87 days for initial, 50% and full sprouting), whereas open conditions resulted in the slowest response (28.20, 55.20 and 91.33 days). The faster sprouting in protected environmental conditions is attributed to stable temperature and humidity that support callus development and cambial union. Less number of days taken for sprouting in poly house condition was also recorded by Raghavendra et al. (2011) ^[13] in wood apple, Syamal et al. (2012) ^[20] in guava, Sivudu et al. (2014) ^[18] in mango (Table 1).

Among grafting months, 15th January recorded the minimum sprouting period (21.11, 43.78 and 75.33 days), while 15th August took the longest (29.11, 51.78 and 83.11 days), indicating that winter conditions favor graft establishment by reducing transpiration stress and enhancing healing. The results are substantiated by Roshan et al. (2013) ^[15] in aonla, Abbas et al. (2013) ^[1] in guava, Chalise et al. (2013) ^[4] in acid lime (*Citrus aurantifolia* Blanco), Chander et al. (2016) ^[5] in jamun and Barathkumar (2017) ^[3] in aonla.

The interaction effect revealed that 15th January grafting under poly house (C3M6) was the most effective combination this provides optimal warmth and humidity, promoting callus formation, cambial contact and reducing moisture loss-key factors for early graft success. Whereas 15th August under open conditions (C1M1) resulted in the slowest sprouting (94.67 days). These findings are in agreement with similar trends reported in mango, guava, jamun, wood apple, kiwi and aonla by earlier researchers.

Table 1: Effect of grafting months, growing conditions and their interactions on number of sprouts after grafting

	Days taken for initial sprouting				Days taken for 50 per cent sprouting				Days taken for complete sprouting			
	C ₁	C ₂	C ₃	Mean	C ₁	C ₂	C ₃	Mean	C ₁	C ₂	C ₃	Mean
M ₁	31.67	29.33	26.33	29.11	58.67	52.33	44.33	51.78	94.67	83.33	71.33	83.11
M ₂	30.11	27.22	23.67	27.00	57.11	50.22	41.67	49.67	93.11	81.22	68.67	81.00
M ₃	28.56	24.22	21.33	24.70	55.56	47.22	39.33	47.37	91.56	78.22	66.33	78.70
M ₄	27.33	23.56	19.11	23.33	54.33	46.56	37.11	46.00	90.33	77.56	64.11	77.33
M ₅	26.33	21.56	17.56	21.81	53.33	44.56	35.56	44.48	89.33	75.56	62.78	75.89
M ₆	25.22	21.00	17.11	21.11	52.22	44.00	35.11	43.78	89.00	75.00	62.00	75.33

Mean	28.20	24.48	20.85		55.20	47.48	38.85		91.33	78.48	65.87	
	C	M	C X M		C	M	C X M		C	M	C X M	
S.Em ±	0.12	0.09	0.21		0.12	0.09	0.21		0.13	0.09	0.23	
CD@5%	0.35	0.25	0.61		0.35	0.25	0.61		0.37	0.26	0.65	

Note: DAG- Days after grafting; C- Growing conditions; M- Grafting months; C1- Open condition; C2- Shade house condition; C3- Poly house condition; M1- 15th August; M2- 15th September; M3- 15th October; M4- 15th November; M5- 15th December; M6- 15th January

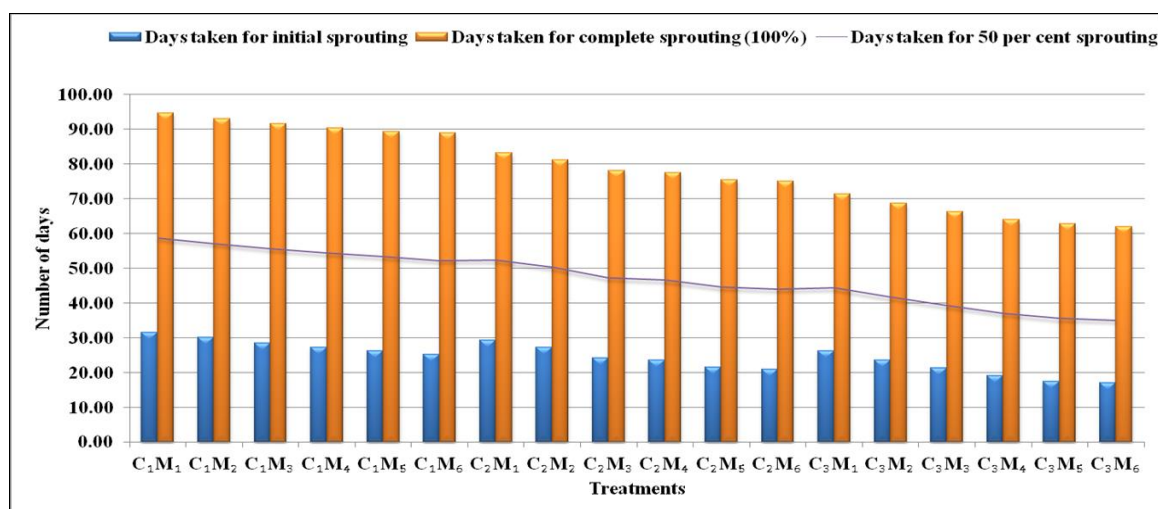


Fig 1: Effect of growing conditions, grafting months and their interaction on days taken for initial, 50 per cent and complete sprouting

- C1M1 - Grafting in the August under open condition
- C2M4 - Grafting in the November under shade house
- C1M2 - Grafting in the September under open condition
- C2M5 - Grafting in the December under shade house
- C1M3 - Grafting in the October under open condition
- C2M6 - Grafting in the January under shade house
- C1M4 - Grafting in the November under open condition
- C3M1 - Grafting in the August under poly house
- C1M5 - Grafting in the December under open condition
- C3M2 - Grafting in the September under poly house
- C1M6 - Grafting in the January under open condition
- C3M3 - Grafting in the October under poly house
- C2M1 - Grafting in the August under shade house
- C3M4 - Grafting in the November under poly house
- C2M2 - Grafting in the September under shade house
- C3M5 - Grafting in the December under poly house
- C2M3 - Grafting in the October under shade house
- C3M6 - Grafting in the January under poly house

3.2 Graft success and graft survival per cent (%)

Significant variations in graft success and survival were observed among different grafting months and growing environments at 120 DAG in acid lime grafted onto Rangpur lime. The poly house condition (C3) consistently achieved the highest success and survival percentages, whereas the open condition (C1) recorded the lowest. Effective graft union formation is largely dependent on healthy callus development between scion and rootstock, which is highly influenced by temperature and humidity. According to Karadeniz (2005) in walnut, high temperature and low humidity in open environments cause desiccation of fragile parenchyma cells forming the callus, hindering graft establishment.

In contrast, poly house conditions provide uniform warmth, higher humidity and protection from external stress, thereby reducing moisture loss, accelerating callus formation, improving vascular connection between scion and stock, and promoting overall healing. Exposure to sunlight, wind and temperature

fluctuations under open conditions leads to dehydration, stress and weak graft union formation. Comparable results were reported in jackfruit (Sulke, 2014)^[19] and guava (Jholgiker et al. (2017)^[7], Nanditha et al. (2017)^[10] and Gotur et al., 2017)^[6]. Maximum graft success and survival were recorded on 15th January (M6), attributable to favorable winter conditions with moderate temperature and reduced disease incidence, which enhance cambial activity and rapid vascular union. Conversely, grafting on 15th September (M2) resulted in the lowest survival due to monsoon-related stresses such as high humidity, temperature variability and greater infection risk. Thus, January offers an optimal period for graft establishment, ensuring better union strength and plant vigor. Similar trends were reported by Panchbhai et al. (2006)^[11] and Roshan et al. (2013)^[15] in aonla, and Uchoi et al. (2012)^[21] in jamun.

The interaction between grafting months and growing conditions significantly influenced graft success and survival in acid lime on Rangpur lime at 120 DAG. The highest performance was achieved on 15th January under poly house conditions (C3M6), as the combination of cool, stable winter weather and a protected, humid microclimate promoted rapid callus formation, precise cambial alignment and strong vascular connectivity. These conditions minimized desiccation and disease stress, ensuring superior graft union. In contrast, grafting on 15th September under open conditions (C1M2) resulted in poor survival due to fluctuating temperatures, high humidity and infection risk. Similar observations were reported in kiwi, aonla, guava and mandarin. Thus, grafting in January under protected structures offers optimal success and long-term vigor.

4. Conclusion

The study confirmed that grafting time and environment significantly influence the success, survival and vigour of softwood grafts in Kagzi lime on Rangpur lime rootstock. Protected conditions, especially polyhouse (C3), ensured better sprouting, callus formation and graft survival due to stable temperature and humidity. Mid-January (M6) was the most suitable period, and its combination with polyhouse (C3M6)

recorded the highest graft success and survival. Hence, softwood grafting of Kagzi lime is best recommended in mid-January under polyhouse conditions to obtain vigorous and true-to-type planting material.

Table 2: Effect of grafting months, growing conditions and their interactions on graft success (90 DAG) and graft survival (120 DAG)

	Graft success (%)				Graft survival (%)			
	C ₁	C ₂	C ₃	Mean	C ₁	C ₂	C ₃	Mean
M ₁	38.89 (38.56)*	55.93 (48.39)	64.44 (53.38)	53.09 (46.77)	65.39 (53.95)	82.09 (64.94)	89.64 (71.19)	79.04 (63.36)
M ₂	34.81 (36.14)	45.19 (42.22)	56.30 (48.60)	45.43 (42.32)	61.46 (51.61)	77.81 (61.87)	88.14 (69.83)	75.80 (61.10)
M ₃	49.26 (44.56)	61.11 (51.40)	67.78 (55.39)	59.38 (50.45)	72.77 (58.53)	83.58 (66.07)	90.15 (71.68)	82.17 (65.43)
M ₄	55.19 (47.96)	64.44 (53.38)	75.56 (60.35)	65.06 (53.90)	75.82 (60.52)	84.45 (66.75)	91.17 (72.68)	83.81 (66.65)
M ₅	62.96 (52.49)	76.67 (61.10)	85.90 (67.98)	75.19 (60.52)	78.79 (62.56)	86.78 (68.65)	92.30 (73.86)	85.96 (68.36)
M ₆	74.81 (59.86)	85.94 (67.98)	90.74 (72.28)	83.83 (66.71)	82.15 (64.99)	88.40 (70.06)	92.86 (74.47)	87.80 (69.84)
Mean	52.65 (46.60)	64.88 (54.08)	73.46 (59.66)		72.73 (58.69)	83.85 (66.39)	90.71 (72.29)	
	C	M	C X M		C	M	C X M	
S.Em ±	0.25	0.35	0.61		0.12	0.17	0.30	
CD@5%	0.72	1.02	1.77		0.35	0.49	0.86	

Note: DAG- Days after grafting; C- Growing conditions; M- Grafting months; C1- Open condition; C2- Shade house condition; C3- Poly house condition; M1- 15th August; M2- 15th September; M3- 15th October; M4- 15th November; M5- 15th December; M6- 15th January; * Figures in parenthesis are arcsine transformed values and are significant (P = 0.05).

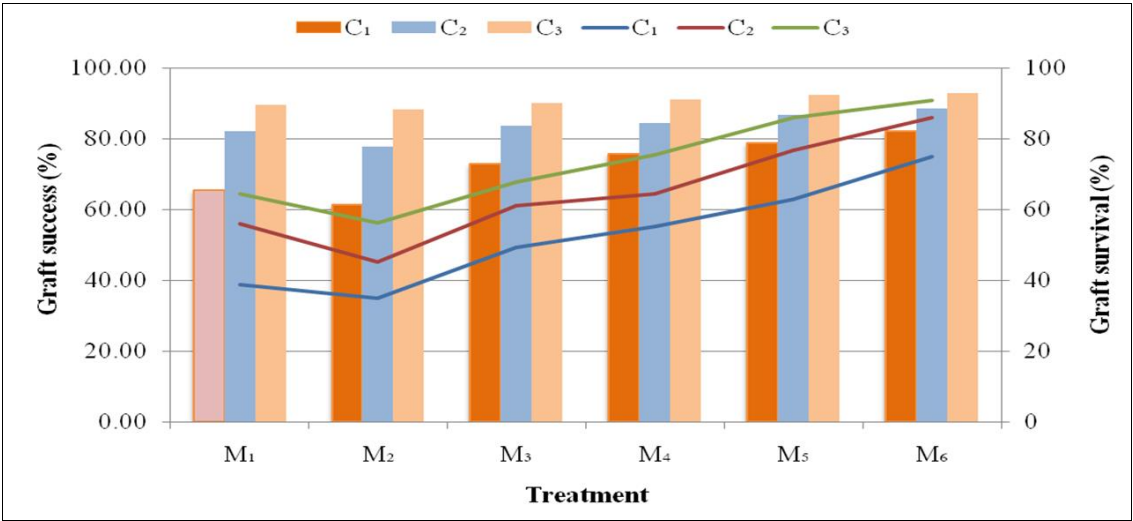


Fig 2: Effect of growing conditions, grafting months and their interaction on graft success (90 DAG) and graft survival (120 DAG)

C1- Open condition M1- 15th August M4- 15th November
C2- Shade house condition M2- 15th September M5- 15th December
C3- Poly house condition M3- 15th October M6- 15th January

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