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Rootstock induced changes in tree physiology and antioxidant enzymes activity in lemon cv. Kagzi Kalan

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ABSTRACT

Lemon cultivar Kagzi Kalan was evaluated on eight rootstocks under Delhi conditions during 2013-14. Rootstocks significantly affected all the physiological and biochemical parameters. Higher transpiration rate (*E*) and stomatal conductance (g_s) were observed in lemon trees budded on sour orange, *Jatti khatti (C jambhiri)*, Troyer citrange (*C. sinensis* x *Poncirus trifoliata*) and rough lemon (*C. jambhiri*) rootstocks. The higher values of photosynthetic rate (*A*) were observed in trees on sour orange and Troyer citrange rootstocks compared to other rootstocks. Lemon trees budded on *Billikhichlli* (*C. reshni*) and *Attani*-2 (*C. rugulosa*) had the highest intrinsic water use efficiency (WUEi), while it was lower in trees budded on *Jatti khatti* and rough lemon rootstocks. Higher relative water control (RWC) was recorded in trees on Troyer citrange and sour orange rootstocks, while highest excised leaf water loss (ELWL) was observed on sour orange followed by trees on rough lemon and Troyer citrange rootstocks. Trees on *Billikhichlli* rootstock had the highest superoxide dismutase (SOD) activity. However, the highest catalase (CAT) activity was found in the leaves of Kagzi Kalan on *Jatti khatti*. Leaves of Kagzi Kalan had the highest glutathione reductase (GR) activities on *Karna khatta* rootstock. The lowest values *E*, g_s, ELWL, and activities of SOD, CAT and GR were observed in the leaves of Kagzi Kalan lemon on *Attani*-2 rootstock.

Key words: Antioxidant enzymes, intrinsic water use efficiency, lemon, photosynthetic rate, relative water content, rootstocks.

INTRODUCTION

With the intensification of fruit production due to socio-economic considerations and in the perspective of climate change, the role of rootstocks in commercial fruit production has increased noticeably. Although rootstocks have several applications such as improving fruit quality, imparting adaptability to climatic and edaphic conditions, inducing dwarfing, environmental stress tolerance *etc*. The priorities of rootstock selection in the tropics and sub-tropics have been focussed mainly on vigour management and securing regular, high fruit yields. Poor soil health and / or toxic elements containing irrigation water may cause more ROS generation which are detrimental as excess ROS damage membranes, proteins, chlorophyll and nucleic acid (Apel and Hirt, 3). Rootstock scion combinations that have lower ROS production and greater activities of antioxidant enzymes are potentially better to sustain growth and productivity under changing scenario of climate and soil. Thus, in changing scenario of climate and soil health, it is imperative to investigate the role of rootstocks on physio-chemical alterations on scion cultivars.

Several reports have established the relationships between various physiological parameters of grafted trees and fruit quality (Naor *et al*., 10). These relationships are important as they provide a basis for selecting the best graft combination for particular environmental conditions with high quality fruit production. Furthermore, in citrus the effect of rootstocks on physiological and biochemical aspects influencing the plant development, productivity and environmental resistance are well documented in Valencia orange (Kaplankıran and Tuzcu, 9). Hence, selection of an appropriate graft combination is very crucial for the production of commercial citrus species. Lemon (*Citrus limon*) is one of the most important citrus fruits worldwide, mostly propagated through budding. *Jatti khatti* (*Citrus jambhiri*), *Karna khatta* (*C. karna*) and rough lemon (*C. jambhiri*) are frequently used rootstocks for lemon cultivars in India. Besides, several lesser known indigenous species, *viz*., *C. rugulosa* and citrus variants frequently found in mid hills of Himalayas are also used as rootstock. The objective of present study was to ascertain the role of rootstocks in alteration of physiological and biochemical parameters of lemon cv. Kagzi Kalan.

MATERIALS AND METHODS

Budded plants were transplanted in a commercial Kagzi Kalan lemon orchard on eight rootstocks (Table 1) at Experimental Orchard in the Division of

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Table 1. Rootstocks used in the experiment.

Common name	Botanical name
Rough lemon	*Citrus jambhiri* Lush
Attani-2	*Citrus rugulosa* (Accn. No. IC 285453)
Jatti khatti	*Citrus jambhiri* Lush
Billikhichlli	*Citrus reshini* Hort. ex Tan.
Sour orange	*Citrus aurantium* L.
RLC-4	*Citrus jambhiri* Lush (Accn. No. IC 274693)
Karna khatta	*Citrus karna* Raf.
Troyer citrange	*Citrus sinensis* x *Poncirus trifoliata*

Fruits and Horticultural Technology, ICAR-IARI, New Delhi in July 2010. The experimental site falls under trans-Gangetic plains of agro-climatic zones of India located at 77°12 'E longitude, 28°40 'N latitude and an altitude of 228.6 m above mean sea level. It has typical subtropical climate characterized by hot and dry summer followed by cold winter. May and June are the hottest months with the maximum temperature ranging between 41 to 44°C, and December and January are coolest months, with the minimum temperature ranging between 3 to 7°C. Trees on eight rootstocks were established at 4.5 m × 4.5 m distance. The experiment was externally guarded on all sides and located within a commercial planting. Soil type was a virgin inceptisol (alluvial soil) with a pH of 7.4 and $EC_{(1:2)}$ of 0.75 dS m^{-1}, a cation exchange capacity (CEC) 7.54 - 10.72 cmol kg^{-1}, organic carbon 0.48%, soil N, 240.23 kg/ha, P_2O_5, 58.65 kg/ha, K_2O 555.92 kg/ha.

Flood irrigation with water (E.C. 1.0-1.30 dS m^{-1}) was utilized during the first two years, and later was changed to a drip irrigation system at 6 l h^{-1} of water per tree, during 4-6 h, thrice a week. The experimental plants were applied 30 kg farmyard manure, 400 g N, 200 g P_2O_5 and 400 g K_2O per tree per year. An annual application of farmyard manure was done in January and urea, single superphosphate, and potassium sulphate were applied one month after flowering. Other cultural operations were carried out uniformly. Foliar micro-nutrients application and pest and disease management were performed in accordance with normal commercial practices.

Five fully developed leaves were collected from each of the selected trees at the middle of the tree four days after irrigation (drip), kept in properly sealed polythene bags. The bags then placed in ice box and brought into laboratory. These leaves were used to determine relative water content (RWC) and excised leaf water loss (ELWL). For ELWL, fresh weight was recorded using electronic balance. Leaf samples were left on working table for two hours at room temperature, thereafter the weight of wilted leaf samples was recorded. ELWL was calculated according to Barrs and Weatherley (4). Net CO_2 assimilation rate (*A*), stomatal conductance (g_s) and transpiration (*E*) were measured on five matured leaves from each treatment using an infrared gas analyzer (IRGA) (LI-6200, LI-COR Biosciences, Lincoln, NE, USA). Intrinsic water use efficiency (WUE_i) was calculated as the ratio of A to g_s (During, 5).

For antioxidant enzymes activities, 10 leaves from each treatment were collected freshly in ice box on 25th October and washed immediately with tap water followed by distilled water. One g of sample was weighed and homogenised in pre-chilled mortar and pestle by adding 5 ml chilled phosphate buffer (50 mM; pH 7.0). The homogenate was collected in oak-ridge tubes and centrifuged at 15,000 x g for 20 min at 4°C. The supernatant so obtained was sieved through two layers muslin cloth and stored in refrigerator which was used as extract for the estimation of following antioxidant enzymes. Soluble protein content of the leaf samples was determined according to Lowry *et al.* (8). The superoxide dismutase (SOD activity in leaf samples was measured according to Fridovich (6). The absorbance of each mixture was then read at 560 nm using a UV-VIS double-beam PC 8 scanning Auto-cell spectrophotometer (UVD-3200, Labomed Inc., Culver city, CA, USA). The complete reaction mixture without added enzyme extracts gave the maximum colour and served as a control. However, catalase (CAT) activity in each plant sample was measured according to Luck (9). Residual H_2O_2 was estimated by titrating the reaction mixture against 0.01M $KMnO_4$ until a faint pink colour persisted for at least 15 sec. Glutathion reductase (GR) was assayed according to Smith *et al.* (13). The reaction was initiated by adding 0.1 ml of 2.00 GSSG (oxidized glutathione) and the increase in absorbance at 412 nm was recorded at 25°C over a period of 10 min. on a on UV-VIS double beam PC 8 scanning Auto cell spectrophotometer, UVD 3200 (Labomed, Inc, USA). Experiment was conducted in complete randomised block design (CRBD) with five replications. Data were analysed using analysis of variance OPSTAT, HAU, Hisar, Haryana (India). P values ≤ 0.05 were considered as significant.

RESULTS AND DISCUSSION

There were significant difference ($P \leq 0.05$) in transpiration rate (*E*), photosynthetic rate (*A*), and stomatal conductance (g_s) of Kagzi Kalan lemon among the rootstocks tested (Table 2). Significantly higher *E* rate was measured on sour orange rootstock which was found non-significant with trees on *Jatti* *khatti* and Troyer citrange rootstocks. Amongst all scion rootstock combinations, the highest values of '*A*' was observed in Kagzi Kalan trees on sour orange, which was non-significant with trees on Troyer citrange rootstock as compared trees on other rootstocks. Furthermore, values of 'g_s' were also observed higher on sour orange, Troyer citrange, and *Jatti khatti* rootstocks as compared to trees on other rootstocks. The lowest value of '*E*' and 'gs' were observed in trees on *Attani*-2 rootstock, while, '*A*' was found lowest on RLC-4 rootstock which was not differed significantly with trees on *Attani*-2 rootstock. Rootstock also significantly influenced intrinsic water use efficiency (WUEi) of lemon cultivar Kagzi Kalan (Table 2). Trees grafted on *Attani*-2 rootstock had the highest WUE_i of scion cultivar which was non-significant with trees on *Billikhichlli*, kanna khatta and Troyer citrange rootstocks. Significantly lower values of A/g_s were observed in trees grafted on *Jatti khatti* and rough lemon rootstocks. Rootstock also influenced relative water content (RWC) and excised leaf water loss (ELWL) of lemon trees significantly (Table 2). Higher values of RWC was observed in trees on Troyer citrange, which was statistically non-significant with trees on sour orange, *Billikhichlli* and *Attani*-2 rootstocks, while significantly lowest RWC was found on *Jatti khatti* rootstock. However, significantly highest ELWL was observed on sour orange rootstock followed by trees on rough lemon rootstock which was non-significant with trees on Troyer citrange rootstocks. Plants maintain a balance between carbon assimilation, storage and growth in response to development and environmental signals (Smith and Stitt, 14). Moreover, fruit set and further vegetative and fruit development in citrus are supported mainly by actual photosynthetic rates (Syvertsen and Lloyd, 15). In our study, leaves of lemon Kagzi Kalan budded on sour orange and Troyer citrange exhibited higher values of *A* and g_s than other rootstock-scion combinations at higher RWC. However, trees on *Attani*-2, *Billikhichlli* and Troyer citrange had the higher WUE_i. As the scion cultivar was same, differences in parameters might be due to a root-derived gradient, which were also reported in citrus (Rodriguez- Gamer *et al.*, 12) as well may be because of variations in cell size due to rootstock effect, which favours cell to cell water exchange. These findings are in agreement with those of grapevine (Naor *et al.*, 10). Indeed it was observed that fresh detached leaves of lemon trees either on *Attani*-2 or RLC-4 had less ELWL than trees on other rootstocks might be due to role of these rootstocks in maintaining thicker leaf cuticle of lemon scion. Numerous studies have also shown that thicker cuticle is associated with more limited leaf water loss, and thus advantageous to plants. Furthermore, we found higher RWC and lower '*E*' and ELWL in Kagzi Kalan trees either on *Attani*-2 or *Billikhichlli* suggesting ability of these combinations to sustain better growth and yield under the conditions of limited water availability. It was also reported that lower ELWL, *E* and higher RWC in plant leaves could be considered as selection criteria to breed plant against drought conditions (Rahman *et al.*, 11).

The superoxide dismutase (SOD) and catalase (CAT) activities also varied significantly ($P \leq 0.05$) on different rootstocks (Fig. 1A, B). Trees of Kagzi Kalan lemon on *Billikhichlli* rootstock had the highest SOD activity followed by trees on rough lemon and *Jatti khatti* rootstocks. The lowest SOD activity in leaves of Kagzi Kalan was observed on *Attani*-2 rootstock. The highest CAT activity was found in leaves of Kagzi Kalan on *Jatti khatti* followed by *Billikhichlli* which was non-significant with trees on sour orange

Table 2. Effect of rootstocks on transpiration rate (*E*), stomatal conductance (g_s), photosynthetic rate (*A*), intrinsic water use efficiency (WUE i), relative water conduct (RWC) and excised leaf water loss (ELWL) of lemon cv. Kagzi Kalan.

Rootstock	*E* (mol H_2O m^{-2} s^{-1})	g_s (mol H_2O m^{-2} s^{-1})	*A* (μ mol m^{-2} s^{-1})	WUE_i (μ mol CO_2 mol^{-1} H_2O m^{-2} s^{-1})	RWC (%)	ELWL (%)
Rough lemon	4.14	0.107	6.98	65.79	71.99	18.98
Attani-2	2.69	0.053	5.22	98.07	77.00	13.89
Jatti khatti	4.58	0.120	7.28	60.94	66.45	16.24
Billikhichlli	3.19	0.080	7.27	91.61	77.40	14.92
Sour orange	5.06	0.133	11.10	83.75	79.63	20.58
RLC-4	2.79	0.060	4.90	82.83	73.28	14.47
Karna khatta	3.67	0.083	7.40	89.01	70.29	17.18
Troyer citrange	4.55	0.113	10.30	90.80	82.17	18.54
LSD ($P \leq 0.05$)	0.69	0.02	1.02	14.49	1.56	1.43

rootstocks. Trees on *Attani*-2 rootstock had the lowest CAT activity. Notwithstanding, glutathione reductase (GR) activity was also affected significantly ($p \leq 0.05$) amongst rootstock-scion combinations (Fig. 1C). Activity of GR was found the highest when Kagzi Kalan lemon budded on *Karna khatta* followed by trees on *Jatti khatti* and RLC-4 rootstocks. The lowest GR activity was observed in leaves of Kagzi Kalan lemon on *Attani*-2 rootstock. There was also significant difference ($p \leq 0.05$) in total soluble protein content in leaves of Kagzi Kalan lemon on different rootstocks (Fig. 1D). Significantly maximum total soluble protein was recorded on rough lemon rootstock which was non-significant with sour orange, *Karna khatta* and Troyer citrange rootstocks. The lowest soluble protein content was observed on *Attani*-2 rootstock. The antioxidant enzymes could increase the ability of stress tolerance in scavenging ROS and, therefore, higher activity of these enzymes could increase the ability of stress tolerance and delay the senescence (Alscher *et al*., 2). The results of present study showed that Kagzi Kalan lemon trees had higher SOD on *Billikhichlli* suggesting trees on this rootstock could be more potential to remove O_2^- by catalysing its dismutation. However, lemon trees on *Jatti khatti* had higher CAT activity indicated higher H_2O_2 scavenging capacity by this combination. In our study, GR activity was higher on *Karna khatta* and *Jatti khatti* suggesting higher ability of these rootstock-scion combinations for ROS scavenging. Variation in antioxidant enzymes activities in different citrus rootstock species had also been reported by many workers (Almansa *et al*., 1).

In conclusion, our study indicate that rootstock genotypes influenced physiological and biochemical parameters of scion cultivars. *Attani*-2 and RLC-4 could be better rootstock under limited irrigation as lemon on these rootstocks had lower values of

CD (p ≤ 0.05) = 1.03

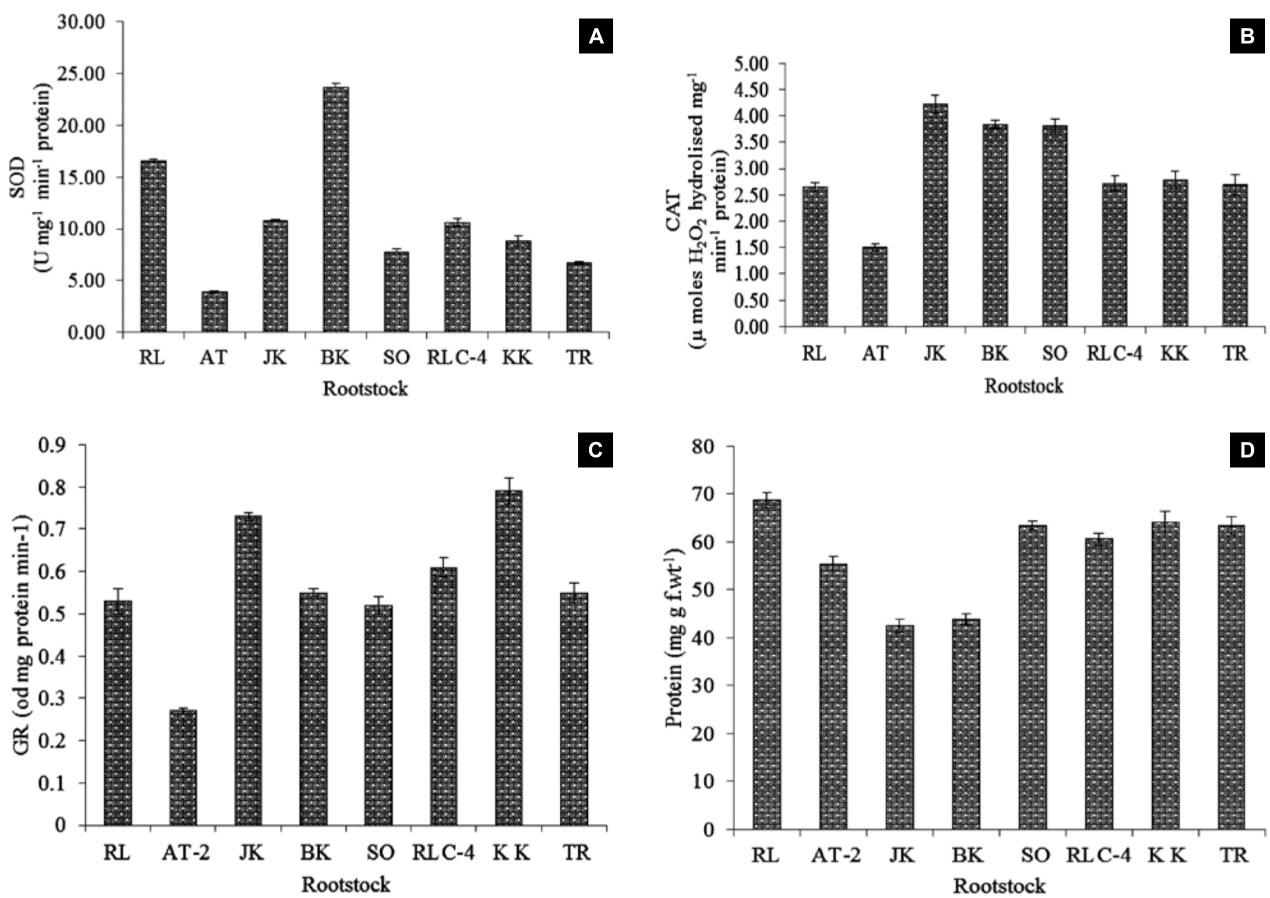

Fig. 1. Influence of rootstocks on superoxide dismutase (A), catalase (B), glutathion reductase (GR), (C) and protein content (D) in lemon cv. Kagzi Kalan trees. Vertical bar represent mean values of five replicates ± S.E. RL = rough lemon; AT = *Attani*-2; JK = *Jatti Khatti*; BK = *Billikhichlli*, SO = sour orange; RLC-4 = rough lemon collection 4; KK = *Karna khatta*; TR = Troyer citrange.

E, ELWL and higher RWC, while sour orange and Troyer citrange may be suitable rootstocks under normal soil moisture conditions because these rootstock had higher values of *E*, g_s, *A*, RWC and *ELWL*. Furthermore, trees on *Jatti khatti*, *Karna khatta* and *Attani*-2 may have higher capacity for ROS scavenging under irrigation with water having higher salt concentrations.

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