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PHYSIOLOGICAL CHANGES DURING FLOWER BUD OPENING IN ROSE (*ROSA HYBRIDA* L.)

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SUMMARY

The present study was undertaken to understand the physiological basis of flower bud opening in rose (*Rosa Hybrida* L.). Flower stems of two cultivars of rose 'Grandgala' and 'First Red' were harvested at six developmental stages (stage 1 to stage 6). The flower stems were kept in water contained in plugged water bottles and water uptake by stems was recorded after 72 hrs. Petals were separated from seven different petal whorls in flowers (outermost to innermost) of both the cultivars at all developmental stages and their fresh weight, turgid weight, RWC & dry weight determined. It was observed that during the opening of flower bud in vase, water uptake was found to be maximum at stage 4 and thereafter, a significant decline was observed with the visible sign of petal rolling and flower wilting. A striking behavior of petal RWC was noticed throughout the flower bud opening. After a peak at stage 4 petal RWC decreased with time and reached to a lowest level (55%) at stage 6. Water potential of flower head followed a parallel increase with flower bud opening. A general increase in fresh weight of petals was recorded during first three phases of flower bud opening followed by a declining trend at later stages. The initial development of flower bud was accompanied by the substantial increase in the dry matter of petals up to stage 3, when the flower bud was attached to the plant. During further opening in vase, the dry matter of the petals tended to fall with the expansion of petals in different whorls.

Key words: **Dry weight, fresh weight, relative water content, water potential, water uptake.**

INTRODUCTION

The postharvest life of many flowers comprises of a period during which the flower opens from the bud or near-bud stage. New techniques for handling of cut flowers requires a better understanding of bud harvest, storage and the mechanism of bud opening. The mechanisms of bud opening vary for different flowers and are sensitive to various environmental influences, notably temperature, water relations, carbohydrate supply and light. Maintenance of optimal water balance is a fundamental objective of cut flower handling. Water potential of cut flower tissue is, in turn, affected by water loss due to transpiration, and also by process leading to cell enlargement, especially growth of the flower petals during flower opening. Van Doorn *et al*. (1991) suggested that the poor opening of cut 'Madelon' roses was initially due to relatively low levels of carbohydrate reserves in the corolla, whereas at a later stage it was partially due to a low water potential. Petal expansion involved rhythmic increase in fresh and dry weight. Weinstein (1957) reported that the fresh weight of "Better Times" rose petal increased until the flower was fully open, but the petal dry weight decreased. These

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data are consistent with an opening mechanism involving cell expansion without an increase in cell number. The fresh weight: dry weight ratio (Fw/Dw) is an indirect measure of the portion of the dry weight that consists of osmotically active solutes. The Fw/Dw of each petal whorl increased with time (Evans and Reid 1988) and enhanced rapidly as the petals in the outer whorl expanded. Petals from the inner whorl, which would not be expanding during that time, showed only a slow increase in fw/dw. In cut roses, leaves contribute mainly for water losses through transpiration (Urban *et al.* 1994). A developmental study was therefore conducted to study the physiological changes during flower bud opening in roses.

MATERIALS AND METHODS

Cut-roses Grandgala and First Red (*Rosa hybrida* L. cultivars) were obtained from a commercial grower (German Garden), Dharuheda, Haryana, India. Flower stems were harvested at the following different developmental stages: Stage 1, closed and pigmenting buds; Stage 2, bud still closed and heavily pigmented; Stage 3, flowers with outer petal whorl just unfurled (commercial stage); Stage 4, flowers 3d after harvest; Stage 5, flowers 6d after harvest; Stage 6, flowers 9d after harvest in vase. Petals were harvested from seven different outermost to innermost whorls in each flower in 'Grandgala' and 'First Red' from all developmental stages. The first three stages represent the attached bloom, while the next three stages were maintained in vase. Flowers were kept in distilled water, for 1 to 9d at $20^0 \pm 2^0$C and a relative humidity of 65 ± 5 %. The room was illuminated (25 µmol m^{-2} s^{-1} light intensity) for 12 hr using cool white fluorescent tubes. Stage 3 flower stems were re-cut under water to a stem length of 60 cm with three leaves for all experiments.

Flower stems were kept in 250 ml bottles holding 150 ml of water. The mouth of the flask was plugged with non-absorbent cotton plug, to reduce water loss. The amount of water taken up by the flower stem was recorded after 72 hr. The vase water was changed every third day.

Relative water content of petal was calculated as described by Weatherley (1965). The petal discs (5 cm in size) were taken and weighed immediately to determine the fresh weight. The petal discs were then floated on distilled water for about 4 hrs at room temperature in the diffused light. The turgid petal discs were weighed and dried at 65°C in an oven to a constant dry weight. The RWC was calculated by the following formula

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

The water potential of the flower head was determined by using pressure chamber method as described by Scholander *et al.* (1965). The flower bud stalk was cut at 5 cm below the pedicle and placed in the sample holder of the pressure chamber. The pressure required for appearance of water at the cut end of the bud stalk was recorded.

The petal whorls of the flowers were separated and then the fresh weight of these petal whorls recorded. The same samples were then oven dried at 60°C in an oven and their dry weights were determined. Statistical analysis was carried out using factorial CRD (complete randomised design) (Gomez and Gomez 1984). In all the experiments there were fifteen flowers per treatment and each treatment had three replications. The data obtained from observations were subjected to ANOVA and critical difference (CD) was calculated at $P = 0.05$.

RESULTS

Water uptake was found to be maximum at stage 4 and thereafter, a significant decline was observed with the visible sign of petal enrolling and flower wilting (Table 1). During the first three days in vase, 'Grandgala' showed a higher uptake of water than 'First Red' but during later stages there was a severe decline in water uptake at stage 5 and stage 6. Similar trend of water uptake was also observed in 'First Red' but the decline in water uptake was not as severe as recorded in 'Grandgala'. The flower stems of 'First Red' showed 25 percent and 53 percent more water uptake at stage 5 and 6 respectively in contrast to flower stems of 'Grandgala'.

Table 1. Water uptake (mL/72h) in rose varieties 'Grandgala' and 'First Red' at different stages of bud opening.

Stages	'Grandgala'	'First Red'
S4	40.6	38.1
S5	24.6	30.7
S6	15.2	23.4
Critical difference at P = 0.05		
Variety (V)	1.15	
Stage (S)	1.40	
Variety x Stage	1.99	

Petal RWC showed a continuous rise in all the petal whorls of both cultivars up to stage 4, from a level of 72 percent to a higher level of 86 per cent (Table 2). After a peak at stage 4 petal RWC decreased with time and fell to a lowest level (55%) at stage 6. 'First Red' maintained significantly higher RWC compared to 'Grandgala'. Critical differences in petal RWC were also encountered in outermost and innermost petal whorl.

Water potential in flower head followed a parallel increase with flower bud opening (Table 3). From an initial level of –7.2 to –6.1 bars at stage 1, when the flower bud was tightly closed, it exhibited a sharp

Table 2. Relative water content (%) in petals of varieties 'Grandgala' and 'First Red' at different stages of bud opening.

Treatments	Stages						Mean
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	
'Grandgala'							
W1	72.03	78.83	85.27	87.00	65.57	54.90	**73.93**
W2	73.97	77.47	84.13	86.08	67.43	57.33	**74.40**
W3	72.13	77.87	83.13	86.19	70.53	60.87	**75.12**
W4	72.10	74.93	81.40	86.23	70.57	62.53	**74.63**
W5	71.27	74.47	79.93	84.83	71.20	63.20	**74.15**
W6	70.37	72.57	79.20	84.60	71.90	73.63	**75.38**
W7	70.97	70.83	76.20	82.30	70.27	64.00	**72.43**
Mean	**71.83**	**75.28**	**81.32**	**85.32**	**69.64**	**62.35**	**-**
'First Red'							
W1	73.87	75.50	83.20	89.93	70.73	60.93	**75.69**
W2	73.10	74.17	82.93	89.10	71.27	62.20	**75.46**
W3	72.67	73.83	81.63	87.33	71.40	63.87	**75.12**
W4	72.80	73.33	80.47	86.83	73.47	63.90	**75.13**
W5	71.50	71.07	80.07	86.23	73.73	64.37	**74.50**
W6	70.67	69.93	79.77	85.57	74.67	64.80	**74.24**
W7	71.17	69.43	78.97	84.80	73.60	63.57	**73.59**
Mean	**72.25**	**72.47**	**81.01**	**87.11**	**72.70**	**63.38**	**-**
Grand mean	**72.04**	**73.87**	**81.16**	**86.22**	**71.17**	**62.86**	**-**

Critical difference at P = 0.05

Variety (V)	0.35	S1: Closed bud
Stage (S)	1.05	S2: Closed and heavily pigmented bud
Whorl (W)	1.22	S3: Commercial stage
Variety x Stage	2.10	S4: Flower 3 days after harvest
Variety x Whorl	2.44	S5: Flower 6 days after harvest
Stage x Whorl	7.33	S6: Flower 9 days after harvest
Variety x Stage x Whorl	NS	W1: Outermost petal whorl, W7: Innermost petal whorl

Table 3. Flower head water potential ($\psi_{w,}$ bars) in rose varieties 'Grandgala' and 'First Red' at different stages of bud opening.

Stages	'Grandgala'	'First Red'
S1	-7.20	-6.13
S2	-4.37	-5.30
S3	-3.91	-4.35
S4	-3.65	-2.76
S5	-12.08	-10.47
S6	-14.18	-11.92
Critical difference at P = 0.05		
Variety (C)	0.159	
Stage (S)	0.276	
Variety x Stage (VxS)	0.390	

increase and marked the peak at –3.7 to –2.8 bars during stage 4, when flower bud started opening. Thereafter, a significant decline was registered at stage 5 and 6 with visible petal senescence. The flower head of 'First Red' maintained higher water potential at all the stages except at stage 2 and 3 in contrast to variety 'Grandgala'.

An increase in fresh weight of petals was recorded during first three phases of flower bud opening followed by a decline at later stages (Table 4). But this decline in fresh weight was found to be whorl specific in both the cultivars. Outermost petal whorl (W1) lost their fresh weight earlier than innermost. As apparent from the data, the outermost petal whorl of 'Grandgala' lost its fresh weight by about 8 per cent at stage 4 and 27 per cent at stage 5. Simultaneously innermost whorl (W7) in 'Grandgala' reflected a gain in fresh weight of 3 per

Table 4. Fresh weight (mg whorl^{-1}) of rose petals in varieties 'Grandgala' and 'First Red' at different stages of bud opening.

Treatments	Stages						Mean
	S1	S2	S3	S4	S5	S6	
'Grandgala'							
W1	0.518	0.924	1.524	1.402	1.108	0.947	**1.071**
W2	0.494	0.850	1.391	1.355	1.511	1.012	**1.102**
W3	0.450	0.836	1.350	1.354	1.405	1.112	**1.085**
W4	0.395	0.792	1.288	1.303	1.349	1.146	**1.046**
W5	0.378	0.755	1.125	1.246	1.300	1.015	**0.970**
W6	0.310	0.693	1.084	1.114	1.137	1.016	**0.892**
W7	0.298	0.652	0.987	1.014	1.078	1.013	**0.840**
Mean	**0.406**	**0.786**	**1.250**	**1.255**	**1.270**	**1.037**	-
'First Red'							
W1	0.486	0.690	1.029	1.248	1.027	0.900	**0.897**
W2	0.474	0.652	0.986	1.325	1.505	1.225	**1.028**
W3	0.461	0.559	0.852	1.107	1.413	1.227	**0.937**
W4	0.446	0.491	0.790	1.005	1.354	1.107	**0.866**
W5	0.383	0.431	0.757	0.996	1.263	0.995	**0.804**
W6	0.364	0.397	0.711	0.856	1.098	0.955	**0.730**
W7	0.319	0.380	0.674	0.815	0.995	0.870	**0.676**
Mean	**0.419**	**0.514**	**0.828**	**1.050**	**1.236**	**1.040**	-
Grand mean	**0.413**	**0.650**	**1.039**	**1.153**	**1.253**	**1.039**	-

Critical difference at P = 0.05

Variety (V)	0.005	S1: Closed bud
Stage (S)	0.016	S2: Closed and heavily pigmented bud
Whorl (W)	0.019	S3: Commercial stage
Variety x Stage	0.033	S4: Flower 3 days after harvest
Variety x Whorl	0.038	S5: Flower 6 days after harvest
Stage x Whorl	0.114	S6: Flower 9 days after harvest
Variety x Stage x Whorl	NS	W1: Outermost petal whorl, W7: Innermost petal whorl

cent at stage 4 and 8.4 per cent at stage 5. These gains were relatively higher in the innermost petal whorl of 'First Red' with 17 per cent rise at stage 4 and 32 per cent at stage 5. Finally at stage 6 all the whorls showed a decline in fresh weight.

The initial development of flower bud was accompanied by the substantial increase in the dry matter of petals up to stage 3, when the flower bud was still attached to the plant (Table 5). During further opening in vase, the dry matter of the petals tended to fall with the expansion of petals in different whorls. Losses in dry matter followed a correlative path with flower bud opening. There was a loss of 14, 11 and 10% dry matter during the stages 4, 5 and 6, respectively in outermost petal whorl of 'Grandgala'. Altogether a reverse pattern was observed in outermost petal whorl of 'First Red' with 6, 10 and 12% losses in dry matter at stages 4, 5 and 6, respectively. A higher amount of dry matter accumulated in outer petal whorls in comparison to inner whorls.

DISCUSSION

The vase life of cut rose flower is often short. The cut flower wilts and floral axis bents just below the flower head which is called "bent neck". Development of such symptoms is considered to be caused by vascular

Table 5. Dry weight (mg whorl^{-1}) of petals in varieties 'Grandgala' and 'First Red' at different stages of bud opening.

Treatments	Stages						Mean
	S1	**S2**	**S3**	**S4**	**S5**	**S6**	
'Grandgala'							
W1	0.077	0.125	0.175	0.150	0.134	0.121	**0.130**
W2	0.069	0.106	0.179	0.165	0.154	0.125	**0.133**
W3	0.062	0.094	0.166	0.163	0.150	0.126	**0.127**
W4	0.055	0.080	0.162	0.160	0.156	0.139	**0.125**
W5	0.051	0.071	0.156	0.155	0.145	0.140	**0.120**
W6	0.047	0.067	0.150	0.149	0.143	0.138	**0.116**
W7	0.179	0.060	0.140	0.140	0.135	0.133	**0.131**
Mean	**0.077**	**0.086**	**0.161**	**0.155**	**0.145**	**0.132**	-
'First Red'							
W1	0.056	0.080	0.117	0.110	0.099	0.087	**0.092**
W2	0.053	0.076	0.116	0.098	0.095	0.079	**0.086**
W3	0.052	0.066	0.096	0.084	0.077	0.068	**0.074**
W4	0.048	0.060	0.085	0.072	0.067	0.062	**0.066**
W5	0.044	0.054	0.084	0.074	0.068	0.062	**0.064**
W6	0.042	0.052	0.074	0.066	0.062	0.058	**0.059**
W7	0.038	0.048	0.073	0.066	0.062	0.057	**0.057**
Mean	**0.048**	**0.062**	**0.092**	**0.081**	**0.076**	**0.068**	**-**
Grand mean	**0.062**	**0.074**	**0.127**	**0.118**	**0.111**	**0.100**	**-**

Critical difference at P = 0.05

Variety (V)	0.004	S1: Closed bud
Stage (S)	0.003	S2: Closed and heavily pigmented bud
Whorl (W)	0.003	S3: Commercial stage
Variety x Stage	0.005	S4: Flower 3 days after harvest
Variety x Whorl	0.006	S5: Flower 6 days after harvest
Stage x Whorl	0.018	S6: Flower 9 days after harvest
Variety x Stage x Whorl	NS	W1: Outermost petal whorl, W7: Innermost petal whorl

occlusion, leading to reduced water uptake at later stages of flower bud opening. Comparatively higher uptake of water in 'First Red' (Table 1) at stage 5 and 6 may be due to higher tolerance to bacteria and thus exhibit slow development of vascular occlusion. Van Doorn *et al.* (1989) reported a positive correlation between the number of bacteria and hydraulic conductivity of the flower stem. During flower bud opening, there was a continuous change in the petal RWC (Table 2). A few days after cutting an increase in petal water content was observed that might be a result of higher uptake of water at stage 4 thereby causing decreased dry matter content (Table 5). When the flower bud reached to maturity a sudden fall in petal RWC occurred due to cavitation in xylem vessels at this stage (Dixon *et al*. 1988). Cavitation in turn will induce greater hydraulic resistance in stem followed by a further lowering of water status, which result in cavitation of more and more xylem vessels. It is likely that a self-perpetuating decline in petal water status will occur. The water potential of flower head showed positive relationship with the developing corolla, exhibiting an increase upto stage 4 and then a decline with the senescence of flower (Table 3). 'Grandgala' and 'First Red' roses placed in water immediately after harvest at stage 3 showed a sharp rise in water potential within 3 days. This enhancement was correlated with the large amount of water uptake by the developing corolla (Table 1). Similarly, the low water uptake (Table 1) at later stages appeared to be the sole cause of decreased flower head water potential, which in turn reduced elongation growth or caused petal wilting at stage 6. Therefore, in cut rose flowers opening was virtually terminated with the fall in water potential and these symptoms appeared much earlier in outer petals.

Flower bud opening of 'Grandgala' and 'First Red' was closely related to the increase in fresh weight (Table 4) of the corollas. Such a relationship between bud opening and fresh weight was also found during the diurnal rhythm of bud opening in cut rose flowers (Van Doorn *et al.*, 1991). Difference in the fresh weight gain in different petal whorls might be due to water loss as outer whorls are exposed to air. It was found that normal development of intact rose flower in polyhouse is accompanied by a substantial increase in dry matter throughout the first three stages of flower bud development. This increase in dry weight (Table 5) suggested that under normal condition the corolla is a strong sink throughout its growth. However, the demand of this sink is normally fulfilled by substrates from the vegetative parts of the intact plant. Cutting caused a gradual loss in dry matter accumulation of cut flowers. It seems to be a direct cause of higher rates of petal expansion accompanied by higher respiratory rates and subsequent decline in starch and sucrose content (Nichols and North 1967). It is reasonable to assume that the supply of dry matter for a growing corolla in the cut flower consisting of only three leaves and a 60-cm stem, as used in these experiments, may be very small. It was not surprising to find that there was a measurable depletion of dry weight in petals.

It can be concluded from the present investigation that rose flower opening is governed by various factors such as cell wall extensibility, water uptake, water potential, relative water content and gain and loss in fresh and dry weight of petals. The changes in the growth rate of rose petals are presumably caused by a change in one or more of these factors. The data presented here is consistent with this hypothesis.

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