Export of Soluble Sugars and Increase in Membrane Permeability of Gladiolus Florets during Senescence

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Summary

Export of soluble sugar, changes in all membrane permeability, and production rate of ethylene in the perianths of the lower florets on the gladiolus spikes were determined as related to physiological events affecting the wilting of perianths after they were fully unfolded.

1. Perianths appeared to be wilting between 2 and 3 days after unfolding fully (DAU). Concurrent with the onset of wilting, perianths rapidly decreased in fresh and dry weights.

2. Between 1 and 2 DAU, one day before perianths started to wilt, total soluble sugar contents began to decrease. Between 2 and 3 DAU, the largest loss of total soluble sugars occurred, most of which was possibly due to export from perianths to other parts. Concurrent with the export of sugars, an increase in leakage of ions from perianth tissues was observed.

3. Rates in ethylene production of perianths did not show a significant change in relation to the time of wilting. Depletion of sugars was discussed as related to the disintegration of membranes, which could cause perianths to wilt.

Introduction

The longevity of gladiolus spikes, like the freesia, may be affected by the development and the rate of ageing of florets (Spikman, 1989). There were several reports on gladiolus floral development and ageing (Bravdo et al., 1974; Burzo et al., 1989; Ferreira et al., 1989; Marousky, 1969; Mayak et al., 1973; Yamane et al., 1991), but information on ageing is limited.

Petal senescence is generally accompanied by the consumption of starch and sugars as respiratory substrates (Borochov and Woodson, 1989; Halevy and Mayak, 1979). Sucrose treatments improved bud development and increase the vase life of cut gladiolus spikes (Bravdo et al., 1974; Marousky, 1969; Mayak et al., 1973). Starch in the perianths on cut gladiolus spikes was exhausted at the early stage of the unfolding process (Yamane et al., 1991). After the perianths had unfolded fully, soluble sugars content decreased. These results suggest that the decrease of soluble sugars could be attributed to their export to other plant parts. Part of the dry matters in the corolla is exported to other organs in some kinds of flowers after pollination (Borochov and Woodson, 1989; Halaba and Rudnicki, 1989; Nichols and Ho, 1975).

Rudnicki et al. (1986) recommended a treatment with silver thiosulfate (STS) and sucrose solutions for postharvest storage of gladiolus spikes. Ferreira et al. (1986) reported that perianths of gladiolus showed a typical climacteric respiration pattern. These data provide a possible relationship between ethylene production and senescence in gladiolus florets.

Senescence of petals was accompanied by a dramatic increase in leakage of potassium and other ions (Borochov and Woodson, 1989; Goszcynska and Rudnicki, 1988; Van Meereren, 1979). Disorganization of cell membranes associated with these leakages brings about a loss of wa-
ter from petal cells, manifested by wilting (Goszczynska and Rudnicki, 1988). Hence, sugars may affect petal longevity by modifying properties of cell membrane (Goszczynska et al., 1990).

The objectives of this paper are to evaluate export of sugars in wilting perianths, to determine the permeability of cell membranes and the production of ethylene as related to senescence of perianths on cut gladiolus spikes.

**Materials and Methods**

**Plant material**

Gladiolus plants (*Gladiolus grandiflorus*), cv. Fujinoyuki, were grown in a greenhouse at Utsunomiya University. Spikes were cut when a perianth of the first floret emerged from bracts on September, 1991. They were transferred immediately to a room which was controlled at 20°C, 75~90%RH and 2.5 μmol·m⁻²·s⁻¹ of continuous white fluorescent light. The stem-end was recut at 110cm in length, leaving 3 to 4 leaves, and re-immersed in deionized water. Florets were fully unfolded under the room conditions, and uniform ones of them were labeled from the first to the sixth floret on the spikes. Seven florets were sampled at 0, 1, 2, 3 and 4 DAU. Since preliminary experiments on an isolated floret showed that a small fraction of dry matter was exported from the perianth to the ovary but not to the stamens and style, perianths of florets with their ovaries removed were used. Perianths with stamens and styles attached were weighed quickly, and each was placed in a 430 ml polypropylene container and sealed with a polyethylene cap, fitted with a silicone rubber plug.

**Measurement of carbon dioxide and ethylene**

After 1 hr of incubation at 20°C, a 1-ml sample of gas was withdrawn from each of 3 containers and analyzed for carbon dioxide concentration by gas chromatography (GC). The GC was equipped with a thermal conductivity detector and a combined column of Porapak Q, Shimalite Q and Molecular Sieve 5A (Shimadzu). After 2 hr, 1 ml of gas was sampled from each of other 3 containers to determine ethylene concentration with the GC equipped with a flame ionization detector and an activated alumina column. No ethylene was detected in samples taken from containers with no florets sealed for 2 hr.

**Measurement of soluble sugars and ion leakage**

After gas analysis, four perianths were lyophilized and weighed. Soluble sugars were extracted from each perianth with 80% ethanol and aliquots of the extracts were analyzed for components using a high performance liquid chromatography equipped with a TSK gel Amide-80 column (Tosoh) (Yamane et al., 1991). The sum of glucose, fructose and sucrose is regarded as total soluble sugars (TSS).

From each outer segment of the other 3 perianths, twenty discs, 10 mm in diameter, were prepared. Ten discs were held at 20°C and the rest was frozen at -20°C in a 50-ml vial for 1 hr. Then 20 ml of 2% mannitol solution was poured into each vial and the vials were shaken for 3 hr at 20°C. Potassium concentration and electric conductivity of the solution were determined with an atomic absorption spectrophotometer and an electric conduct meter, respectively. Ion leakage from fresh discs was expressed as a percentage of that from frozen discs.

**Calculation of daily changes of dry matter components**

Daily loss of dry weight and TSS in the perianth were computed, using the mean values of the previous day's reading as reference base. Loss of carbohydrates by respiration was estimated from the amount of respired carbon dioxide on the assumption that the carbon originated from hexoses. The remainder of daily loss of dry weight after subtraction of the respiratory loss was taken as a result of export and/or import of sugars and inorganic ions.

**STS treatment**

Fifteen florets with the perianths unfolded fully were detached in August, 1991 from cut gladiolus spikes that were held in a room under the same conditions as described above. The cut end of a floret was immersed in STS solution (0.4 mM AgNO₃ plus 1.6 mM Na₂S₂O₃, v/v) and subsequently transferred to a vase containing deionized water. Three groups with 5 florets each were treated with STS solution for 0, 2 and 4 hr.

**Results**

The perianths appeared wilted between 2 and 3
DAU. Associated with the start of wilting, the fresh and dry weights of perianths decreased (Fig. 1).

Carbon dioxide production per perianth was about 0.5 ml·hr⁻¹ in the first 3 DAU and decreased at 4 DAU (Fig. 2). Carbon dioxide production, expressed on unit fresh weight, showed a climacteric rise at 3 DAU (Fig. 2).

Amounts of fructose and glucose in a perianth decreased by 80% between 1 and 3 DAU (Fig. 3). Sucrose contents increased at 2 DAU and decreased afterwards (Fig. 3).

Daily loss of total dry matter was statistically significant between 2 and 3 DAU (Table 1). Daily loss of TSS was apparent between 1 and 4 DAU and contributed to a substantial part of the total dry matter lost (Table 1). Loss of residues was similar to that of TSS, but it was not statistically significant except between 2 and 3 DAU (Table 1). Loss by respiration consistently occurred (Table 1). A loss of dry matter due to export occurred between 2 and 3 DAU, accompanied by losses in TSS and residues (Table 1).

Ethylene production per perianth did not show a significant change for the whole period (Fig. 4). Ethylene production expressed on unit fresh weight increased between 2 and 4 DAU (Fig. 4).

Leakage of potassium and total ions from perianth tissues increased significantly between 2 and 4 DAU (Fig. 5).

STS treatments of florets even for 4 hr had no positive effects on the wilting of perianths (data not presented).

Discussion

Loss of dry matter was clearly shown during senescence of gladiolus perianths (Fig. 1, Table 1).
The reduction in TSS contributed to the substantial dry matter loss with no increase in the residual component. Hence, soluble sugars were considered to have been exported or respired. Between 2 and 3 DAU, the loss of TSS was greater than that by respiration (Table 1). This indicates that export of soluble sugars from perianths occurred. Since the loss of residues can be attributed to hydrolysis of stored and structural carbohydrates and export of inorganic ions, the export of such hydrolyzates could have occurred between 2 and 3 DAU.

Content of reducing sugars in a perianth decreased, but that of sucrose increased before a wilting state became apparent (Figs. 1 and 3). Since acid invertase inhibitor was present in wilted flowers (Borochov and Woodson, 1989; Halaba and Rudnicki, 1989), it is possible that sucrose was prevented from hydrolysis and exported from the perianth to other parts. According to preliminary experiments, a part of the dry matter was exported from a perianth of its ovary. The other part of dry matter could be exported to upper florets on the spikes. $^{14}$C sucrose taken up from the vase solution accumulated to a much higher level in the upper florets than in the lower.
er ones on the gladiolus spikes (Ferreira et al., 1986). This rapid export occurred before wilting began. In carnation (Nichols and Ho, 1975), export of sugars induced by ethylene treatment also started before wilting of the petals became apparent. The loss of sugars as osmotica not only reduces water uptake by perianth tissues (Bravdo et al., 1974), but it may also be accompanied with water loss. Thus, export of sugars is considered to be crucial to the wilting of perianths. The effects of sucrose treatments on longevity of gladiolus florets (Bravdo et al., 1974; Marousky, 1969) may be due to the alleviation of water stress conditions in spikes.

Between 2 and 3 DAU, loss of 45.1 mg of components other than soluble sugars occurred (Table 1). Since ethyl alcohol insoluble residues of a perianth decreased by 35.5 mg between 2 and 3 DAU (data not presented) and starch content was very low in an unfolded perianth (Yamane et al., 1991), a part of the structural components in perianth tissues could have been solubilized and exported.

Van Meeteren (1979) reported that a relationship between water content and leakage of ions in gerbera flowers. Burzo et al. (1989) found that plasmalemma and tonoplast had disintegrated in wilted perianths of gladiolus. In our experiment, leakage of potassium and total ions from perianth tissues increased after 1 DAU, reaching 70 and 80% of maximum 4 DAU, respectively (Fig. 5). The increase in membrane permeability of perianth cells could be caused by the putative decomposition of the structure as discussed above. This, in turn, will decrease ion content in the osmotica and reduce the uptake of water, causing the cells to lose their turgor.

Between 1 and 3 DAU, it was possible that sugars began to be exported from perianth tissues to other parts (Table 1). Concomitantly ion permeability of cell membranes of perianth increased (Fig. 5). Goszczynska et al. (1990) reported that sugar treatments of cut flowers prevented a decrease in phospholipid content of cell membranes in rose petals. If that were the case in gladiolus, export of sugars as well as the consumption by respiration could make the sugar concentrations in the perianth tissues so low that phospholipid contents were not maintained at a level to keep the membrane intact. In this context, the amount of exported sugars was estimated to be comparable to or much more than that consumed by respiration. Physiological effects of export of sugars on the perianth’s senescence remain to be studied.

Ethylene production of perianths during senescence increased when expressed on a fresh weight basis, but not when expressed on the perianth basis. These results suggest that the increase was attributable to water loss from the tissues, and it was not by an increase in enzyme activity. The data agree with the finding that STS treatments do not delay the onset of wilting of detached gladiolus perianths. Beneficial effects of STS treatments before storage at low temperature were reported for the quality of cut gladiolus spikes (Merodio and Plaza, 1989; Rudnicki et al., 1986). Ethylene production by organs other than perianths may affect the senescence of gladiolus spikes.

**Literature Cited**


