

Membrane Competence among and within *Fragaria* Species Varies in Response to Dehydration Stress

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ABSTRACT. The effect of dehydration stress on membrane competence among and within *Fragaria* species was evaluated using index of injury, I_d , and tissue ionic conductance, g_{Ti} . Single accessions of *F. chiloensis* ssp. *lucida* Duch., *F. virginiana* ssp. *glauca* (S. Watson) Staudt, *F. virginiana* ssp. *virginiana* Duch., *F. xananassa* Duch., and *F. vesca* L. were used to study interspecific variation. Leaf thickness and total electrolyte content were greatest for the *F. chiloensis* ssp. *lucida* accession and least for the *F. virginiana* ssp. *glauca* accession, but foliar electrolyte concentration did not vary across accessions. The g_{Ti} values were >5-fold higher from 0 to 2 hours than for other intervals, declining over time. Significant differences in g_{Ti} and I_d values were only evident at 2 and 4 hours within stress levels, and increased as stress level increased. While the *F. chiloensis* ssp. *lucida* accession exhibited low g_{Ti} values at 70% relative water content (RWC), it showed greater relative membrane injury than the other species expressed as g_{Ti} , I_d , or the ratio of stress g_{Ti} to control g_{Ti} as dehydration level increased. Although the *F. virginiana* ssp. *glauca* accession had the highest g_{Ti} values, even at 100% RWC, its relative injury as stress level increased was not as great as that of the *F. chiloensis* accession. In a second experiment, intraspecific variation was examined using four accessions each of *F. chiloensis* and of *F. virginiana* which were dehydrated to 50% RWC. The species mean g_{Ti} , I_d , and g_{Ti} ratio values at 2 and 4 hours for the *F. chiloensis* accessions were lower than those for the *F. virginiana* accessions, but significant intraspecific variation was also observed. In spite of the differences between species means, the evidence of intraspecific variation indicates that not all accessions of a species exhibit similar drought responses (i.e., membrane competence). Due to the consistent conclusions derived from using either g_{Ti} or I_d after 2 or 4 hours of incubation for characterization of membrane competence, g_{Ti} and I_d were comparable techniques for identification of potential drought tolerance in *Fragaria*.

Of the primary progenitor species of cultivated strawberry (*Fragaria xananassa*), accessions of *F. chiloensis*, native to the Pacific coasts of North and South America, are reported to be drought tolerant, and accessions of *F. virginiana*, originating in eastern North America, are reported to be drought susceptible (Darrow, 1966; Davies and Albrigo, 1983; Zhang and Archbold, 1993a, 1993b). Morphological and physiological traits that might contribute to such variation, including stomatal density and position (Darrow, 1966; Davies and Albrigo, 1983; Kelly and Cameron, 1994), cuticle thickness (Archbold, 1993; Davies and Albrigo, 1983), and osmotic adjustment (Zhang and Archbold, 1993a, 1993b), have been proposed, but only the latter has been specifically demonstrated to contribute to greater drought tolerance. Potential intraspecific variation in *F. chiloensis* and *F. virginiana* has not been examined, nor is the drought tolerance of other *Fragaria* species known. There has been a renewed interest in using native *Fragaria* species in cultivar improvement programs (Luby et al., 1991). If drought-tolerant species, or selections within species, could be identified, they would be valuable parental material. A thorough screening for drought tolerance across and within *Fragaria* species is needed. However, an effective and rapid drought-tolerance screening technique needs to be developed. Optimally, the technique should not be destructive of whole plants, be easily used on plant material from a variety of cultural systems, and not be dependent on a time- and space-consuming approach in the field or greenhouse.

Cell membrane competence has been related to drought tolerance in several studies (Leopold et al., 1981; Martin et al., 1987; Premachandra et al., 1991, 1992; Vasquez-Tello et al., 1990;

Whitlow et al., 1992). Membrane permeability to ions may be altered by dehydration stress, perhaps more in drought-susceptible genotypes. Ion or electrolyte leakage from tissue sections into an aqueous incubation solution has been used to indicate cell membrane damage as a result of stress. Solution electrical conductivity (EC) is a measure of ion leakage from tissue, and membrane competence is inversely related to EC of stressed tissues (Blum and Ebercon, 1981; Dexter et al., 1932; Gupta, 1977; Leopold et al., 1981; Ruter, 1993).

Using EC values to distinguish drought-tolerant from drought-susceptible plants is not acceptable because samples from replicate plants may vary significantly in electrolyte content (Stuart, 1939). To obtain a single quantity by which stress effects could be compared across samples, index of injury (I_d) was proposed by Flint et al. (1967). I_d is an expression of tissue injury on a scale of 0 to 100, where 0 is the value of unstressed control tissue and 100 is maximum injury. After stress is applied, an excised tissue piece of uniform dimensions such as a leaf disk is incubated in water and an initial measurement of solution conductivity is made ($EC_{initial}$) using a conductivity probe, often after a 24-h incubation. The tissue is then frozen or autoclaved to completely disrupt membranes and release all ions. Conductivity is again measured after 24 h to obtain maximum ion concentration (EC_{total}). I_d at any incubation time t is defined as

$$I_d = [(R_t - R_o)/(1 - R_o)] \times 100 \quad [1]$$

where $R_t = EC_{initial}/EC_{total}$ for stressed tissues at time t , $R_o = EC_{initial}/EC_{total}$ for nonstressed tissues. This method is preferable to a direct measure of EC because it factors out ion concentration differences among samples. I_d is normalized to control values, allowing comparisons among samples of diverse total ion concentration. I_d , or a variation of it, was reported to effectively identify membrane competence to dehydration stress in woody (Martin et al., 1987) and in herbaceous (Blum and Ebercon, 1981; Premachandra et al., 1991, 1992; Vasquez-Tello et al., 1990) species. It has been criticized because the measurement is taken at an arbitrary time,

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the relationship between I_d and membrane competence has not been determined, and it uses none of the terms commonly used to describe ion movement (Whitlow et al., 1992).

Using estimates of rates of solute leakage from leaf disks may also indicate the effects of dehydration stress. The rate of solute loss from dehydrated leaf disks, measured using a spectrophotometer and expressed as a percentage of total absorption at 280 nm, indicated a positive correlation between the severity of dehydration stress and the rate of leakage. (Leopold et al., 1981). While methods using rates of leakage are reasonable approaches for comparing membrane competence of uniform tissues, a method to compare competence from nonuniform tissues is required. To address these problems, a new estimation of membrane integrity was proposed—tissue ionic conductance, or g_{Ti} (Whitlow et al., 1992). It is based on Fick's First Law of Diffusion and explicitly accounts for tissue thickness, or cut surface area through which the ions pass, and chemical driving force:

$$g_{Ti} = J_j / (A \times (c^i - c^o)) \quad [2]$$

where $J_j = dj/dt$ = the flux of solute species j per unit time, A = the area through which diffusion occurs and is calculated as $2\pi rw$ where r is the radius and w is the thickness of the leaf disk, and $(c^i - c^o)$ = the difference in solute j concentration between the bathing solution (c^o) and the tissue (c^i). Drought acclimated tissue was identified by g_{Ti} values but not I_d values (Whitlow et al., 1992). Tissue dehydrated to 80% of initial fresh mass had consistently higher g_{Ti} values than fully hydrated controls, and tissue that had been previously acclimated to drought conditions had the lowest g_{Ti} values. In these experiments, EC values were converted to $[K^+]$, but it was suggested that ion concentration could be replaced by EC values for calculation of g_{Ti} since they are nearly linearly related and K^+ is the primary ion contributing to EC. The objectives of the following experiments were 1) to compare two methods using electrolyte leakage, I_d and g_{Ti} , for assessing membrane response to dehydration and 2) to determine if membrane competence varies in response to dehydration stress among and within *Fragaria* species.

Materials and Methods

Plant material was obtained from the USDA–ARS National Clonal Germplasm Repository in Corvallis, Ore., as seed or plants and propagated as runner plants. A single accession from each of five species of *Fragaria* were used in Expt. 1; the USDA accession number, if available, and site of origin are in parentheses: *F.*

chiloensis ssp. *lucida* (CFRA 34, California), *F. virginiana* ssp. *glauca* (S. Watson) Staudt (CFRA 281, Utah), *F. virginiana* ssp. *virginiana* Duch. (CFRA 67, Maryland), *F. xananassa* Duch. (CFRA 310, 'Sequoia' parent), and *F. vesca* L. (CFRA 562, Siberia). In Expt. 2, four accessions each of *F. chiloensis* (CFRA 34, California; CFRA 48, Oregon; CFRA 135, Chile; CFRA 24, Columbia) and *F. virginiana* (NCC85-13V, North Carolina; CFRA 98, Montana; CFRA 67, Maryland) were compared. *Fragaria virginiana* ssp. *glauca* (CFRA 281, Utah) was included with *F. virginiana*. The accessions were selected to represent diverse sites of origin.

Plant material was maintained in soilless potting medium (Metro-Mix 360; Scotts-Sierra Horticultural Products, Marysville, Ohio) in 2-L containers in a greenhouse from December to March 1994 under natural light and at a minimum greenhouse temperature of 21 °C. Plants were fertilized weekly with a 20N–20P–20K fertilizer solution containing N at 200 mg·L⁻¹ and watered daily to runoff. Separate plastic bags were placed over entire plants the evening before each experiment to maintain high humidity. Before dawn the following morning, plants were moved from the greenhouse to the laboratory, and ≈20 leaf disks were immediately removed with a 8.6-mm-diameter cork borer from one to two recently mature leaves from one plant of each species. The disks were washed by floating in deionized water for 1 h at ambient temperature. Immediately after collecting the leaf disks, leaf thickness was measured in 10 locations across the sampled leaflets avoiding the midrib and large veins using a digimatic indicator (IDC543; Mitutoyo, Tokyo, Japan). Leaf thickness values were used as indirect measures of cut surface width.

In Expt. 1, leaf disks were removed from the deionized water, blotted dry, and weighed [initial fresh mass (FM)]. Thereafter, disks were weighed at frequent intervals during dehydration on the bench top to 80%, 70%, or 60% of initial FM, requiring up to 2 h. At the appropriate dehydration level, a single disk was weighed (dehydrated FM) and placed in a 13 × 100-mm test tube containing 10 μL water and three 3-mm-diameter glass beads to hold the disk above the water. Preliminary work indicated that the disks maintained a constant water content for up to 2 h in this set up. Excess water was removed from fully hydrated control disks with a paper tissue upon removal from the water, and they were weighed and placed directly in a test tube. After the final disks were ready, deionized water was added to each test tube for a total volume of 7 mL. The test tubes were covered with parafilm and placed in racks on a wrist-action shaker. There were three replicate leaf disks of each accession at each dehydration level. The

Table 1. Mean leaf thickness, total solution EC after freezing and thawing, leaf EC concentration, and relative water content (RWC) after desiccation of accessions representing five *Fragaria* species.

Species (accession no.)	Leaf thickness ^a (mm)	Total EC ^b (μS)	EC concn ^c (μS·g ⁻¹ H ₂ O)	RWC after desiccation to a fresh mass of		
				80%	70%	60%
<i>F. chiloensis</i> ssp. <i>lucida</i> (34)	0.65 a ^w	55 a	3482 ^{ns}	69 ± 5 ^v	61 ± 2	52 ± 1
<i>F. virginiana</i> ssp. <i>glauca</i> (281)	0.21 d	19 d	3468	73 ± 2	67 ± 5	54 ± 2
<i>F. virginiana</i> ssp. <i>virginiana</i> (67)	0.40 c	29 c	3765	69 ± 5	60 ± 1	54 ± 1
<i>F. xananassa</i> (310)	0.50 b	39 b	3780	72 ± 2	66 ± 4	54 ± 3
<i>F. vesca</i> (562)	0.37 c	23 d	3638	73 ± 2	64 ± 3	46 ± 6

^aLeaf thickness, n = 10.

^bTotal EC after freezing and thawing.

^cEC concentration, n = 24. For EC concentration, fresh disk mass minus dry disk mass = g H₂O.

^wMean separation by LSD at $P = 0.05$. Means followed by different letters are significantly different.

^{ns}Nonsignificant.

^vRWC, n = 6, mean ± standard error of mean.

experiment was repeated and the data combined for analysis.

In Expt. 2, intraspecific differences were examined with two treatments, fully hydrated and 60% of initial fresh mass. Otherwise the same procedures were used as in Expt. 1. The experiment was performed once.

The EC of the bathing solution was measured after 2, 4, 6, 9, 12, and 24 h of shaking using a conductivity probe (Amber Sciences Inc., Eugene, Ore.). After 24 h the leaf disks were removed from the test tubes, frozen in liquid N₂, and rapidly placed back into the same test tubes and solutions and shaken for another 24 h. A final EC measurement was taken to estimate the total ion concentration in the leaf disks. Leaf disks were then dried in an oven at 60 °C for 24 h and weighed [dry mass (DM)].

Relative water content (RWC) of each disk was calculated as

$$\text{RWC} = [(\text{dehydrated FM} - \text{DM}) / (\text{initial FM} - \text{DM})] \times 100 \quad [3]$$

I_d and g_{Ti} were calculated using Eqs. [1] and [2], respectively. For g_{Ti} , J_i was calculated as

$$J_i = [(EC_{i+1} - EC_i) \times (7 \times 10^{-6} \text{ m}^3)] / t \quad [4]$$

where EC_i = EC at some time i , and t = the time interval between successive EC measurements. The EC was substituted for the actual ion concentration as suggested by Whitlow et al. (1992). The cut surface area, A , was calculated as described for Eq. [2]. The concentration gradient ($c' - c''$) was calculated as

$$(c' - c'') = [((EC_{\text{total}} - EC_i) \times (7 \times 10^{-6} \text{ m}^3)) / (\text{FM} - \text{DM})] - EC_i \quad [5]$$

with FM as the mass before dehydration treatment, assuming disks rehydrated soon after the start of incubation. The difference of FM – DM was converted from grams to cubic meters of water.

In addition to I_d and g_{Ti} values, the ratio of g_{Ti} at each dehydrated RWC to that at 100% RWC was calculated as a ratio similar to I_d . All data were subjected to analysis of variance (SAS Institute, Cary, N.C.), using least significant difference (LSD) at $P = 0.05$ to compare means when the F values were significant. In Expt. 1, data from both trials were combined, and I_d , g_{Ti} , and g_{Ti} ratio were compared between species within a RWC as well as regressed across RWCs within a species within each time interval. In Expt. 2, *F. virginiana* ssp. *glauca* was included with *F. virginiana* for the purpose of species comparison. The species and accession mean I_d , g_{Ti} , and g_{Ti} ratio values were compared for each time interval by LSD at $P = 0.05$.

Results

EXPERIMENT 1. Leaf thickness varied among the *Fragaria* accessions (Table 1). The *F. chiloensis* ssp. *lucida* accession had the thickest leaves at 0.65 mm, and those of *F. virginiana* ssp. *glauca* accession were thinnest at 0.21 mm. The initial fresh masses of 80%, 70%, and 60% corresponded to ≈70%, 60%, and 50% RWC, respectively. Total solution EC content after freezing and thawing and a 24 h incubation varied considerably among species with *F. chiloensis* ssp. *lucida* 34 the highest and *F. virginiana* ssp. *glauca* 281 the lowest. However, foliar EC concentration was not different among the accessions.

Because general trends for EC over time and differences among accession g_{Ti} values were the same for each dehydration level, only the 50% RWC values are presented. The greatest increase in EC values for all accessions and dehydration treatments occurred in the first interval from 0 to 2 h (Fig. 1A). *Fragaria chiloensis* ssp. *lucida* 34 generally exhibited the highest EC values and *F. virginiana* ssp. *glauca* 281 the lowest. In all dehydration treatments, the g_{Ti} values were highest after 2 h and then declined, as shown for 50%

RWC (Fig. 1B). Also, the range in values among species was greater early and then diminished. For most intervals in the 70% and 60% RWC treatments, differences among accessions were also observed, with *F. virginiana* ssp. *glauca* 281 generally exhibiting higher g_{Ti} values at all RWC's including the fully turgid (100% RWC) samples (data not shown).

The I_d values within each dehydration level generally increased over time, but significant differences among species were only evident at 2 and 4 h (data not shown). At 2 h, I_d values increased as tissue RWC declined (Fig. 2), and the same trends were also observed for the 4-h values but no other interval (data not shown). Although no single accession exhibited the highest I_d value at all three RWC's, the slope of the regression of I_d versus RWC for *F. chiloensis* ssp. *lucida* 34 was more negative than that for the other accessions.

During the 0- to 2-h interval, g_{Ti} values also increased linearly as RWC declined (Fig. 3). While the g_{Ti} values for *F. virginiana* ssp. *glauca* 281 were highest at all RWC's, the slope of g_{Ti} versus RWC for *F. chiloensis* ssp. *lucida* 34 was more negative than that for the other accessions. The g_{Ti} ratio, or the g_{Ti} value at the respective RWC versus the control g_{Ti} value, also exhibited an increase as RWC declined (Fig. 4). Again the greatest change in the g_{Ti} ratio across RWC values was for *F. chiloensis* ssp. *lucida* 281.

EXPERIMENT 2. While there was some intraspecific variation in leaf thickness and EC total values within *F. chiloensis* and *F. virginiana*, the *F. chiloensis* mean values were greater than those

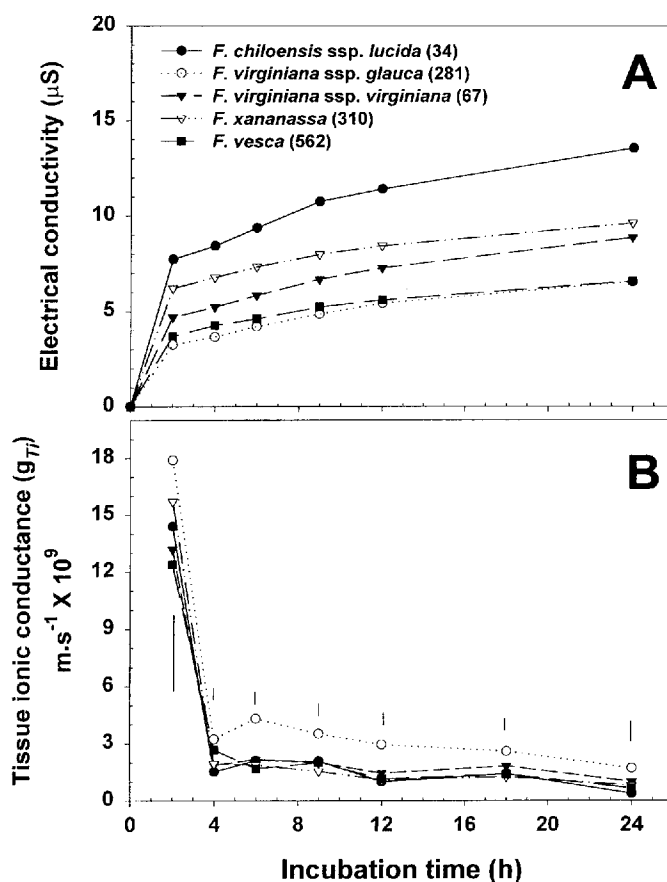


Fig. 1. (A) Electrical conductivity (EC) of five *Fragaria* accessions at intervals over 24 h after leaf disk dehydration to 50% relative water content (RWC). (B) Tissue ionic conductance (g_{Ti}) of five *Fragaria* accessions at intervals over 24 h after leaf disk dehydration to 50% relative water content (RWC). Each data point represents the mean of two experiments, three leaf disks per experiment. For g_{Ti} values, vertical bars represent the least significant difference at $P = 0.05$. Accession numbers are in parentheses.

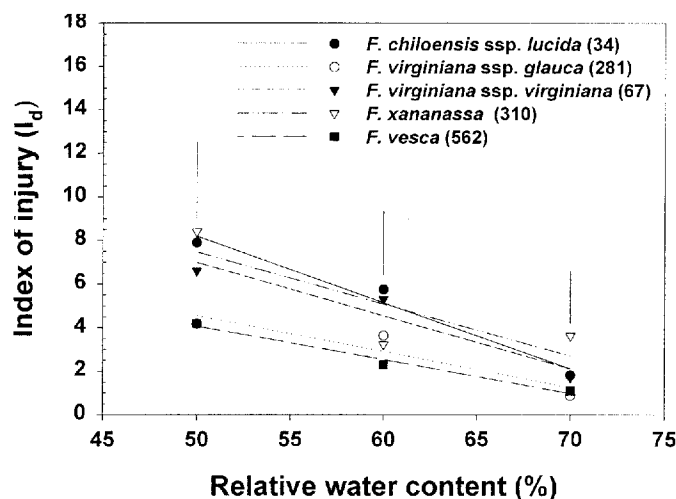


Fig. 2. Index of injury (I_d) of five *Fragaria* accessions at 2 h after leaf disk dehydration versus leaf disk relative water content (RWC). Each data point represents the mean of two experiments, three leaf disks per experiment. Vertical bars represent the least significant difference at $P = 0.05$. The regression equations are *F. chiloensis* ssp. *lucida* 34, (solid line), $I_d = 23.42 - 0.30\text{RWC}$, $r = 0.98$; *F. virginiana* ssp. *glauca* 281, (dotted line), $I_d = 12.83 - 0.17\text{RWC}$, $r = 0.93$; *F. virginiana* ssp. *virginiana* 67, (short dashed line), $I_d = 19.23 - 0.24\text{RWC}$, $r = 0.96$; *F. xananassa* 310, (dash-dot-dot line), $I_d = 19.40 - 0.24\text{RWC}$, $r = 0.83$; and *F. vesca* 562, (long dashed line), $I_d = 11.82 - 0.16\text{RWC}$, $r = 0.99$. All regressions are statistically significant at $P \leq 0.05$. Accession numbers are in parentheses.

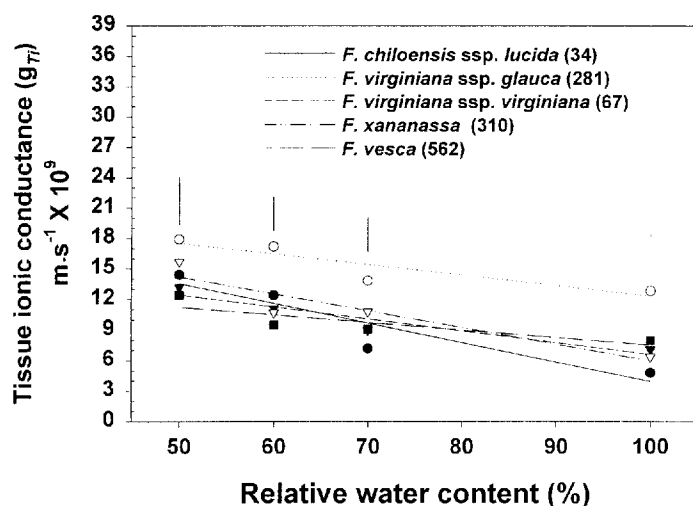


Fig. 3. Tissue ionic conductance (g_T) of five *Fragaria* accessions at 2 h after leaf disk dehydration versus leaf disk relative water content (RWC). Each data point represents the mean of two experiments, three leaf disks per experiment. Vertical bars represent the least significant difference at $P = 0.05$. The regression equations are *F. chiloensis* ssp. *lucida* 34, (solid line), $g_T = 23.10 - 0.19\text{RWC}$, $r = 0.86$; *F. virginiana* ssp. *glauca* 281, (dotted line), $g_T = 22.75 - 0.10\text{RWC}$, $r = 0.81$; *F. virginiana* ssp. *virginiana* 67, (short dashed line), $Y = 18.20 - 0.12\text{RWC}$, $r = 0.90$; *F. xananassa* 310, (dash-dot-dot line), $g_T = 22.35 - 0.16\text{RWC}$, $r = 0.86$; and *F. vesca* 562, (long dashed line), $g_T = 14.92 - 0.07\text{RWC}$, $r = 0.72$. All regressions are statistically significant at $P \leq 0.05$. Accession numbers are in parentheses.

of *F. virginiana* (Table 2). Foliar EC concentration varied within species, but species means did not differ. The respective values for *F. chiloensis* 34 and *F. virginiana* 67 were similar, but not identical, to those in Expt. 1 for the same stress level. Disks in Expt. 2 were dehydrated to $\approx 50\%$ RWC, near the greatest dehydration levels in Expt. 1.

As in Expt. 1, significant differences in g_T and I_d values were only observed at 2 and 4 h. There was significant variation in g_T , I_d , and the g_T ratio values between species and across all accessions

at 2 h (Fig. 5). In *F. chiloensis*, accession 24 exhibited consistently high values, and within *F. virginiana*, NCC85-13V exhibited the highest values. The relative order of the accessions within each species was nearly the same with the g_T ratio as with I_d values. Overall, the species means for g_T , I_d , and g_T ratio of *F. chiloensis*, $17.0 \times 10^{-9} \text{ m.s}^{-1}$, 7.7, and 2.6, respectively, were significantly lower ($P \leq 0.05$) than the *F. virginiana* means at $29.5 \times 10^{-9} \text{ m.s}^{-1}$, 15.7, and 3.2, respectively.

Discussion

The stress levels applied to the disks were similar to those endured by intact leaves in prior studies on whole plant drought tolerance with *Fragaria* (Zhang and Archbold, 1993a, 1993b). Water potential isotherms were developed using excised intact leaves and estimated loss of leaf turgor near 80% RWC for nonstressed *F. chiloensis* and a *F. virginiana* accessions. Loss of leaf turgor for drought-hardened plants of the *F. chiloensis* and the *F. virginiana* accessions occurred at 64% and 80%, respectively. Stress levels in this study ranged from $\approx 50\%$ to 70% RWC (Tables 1 and 2). In the earlier studies the *F. chiloensis* accession, *F. chiloensis* ssp. *pacifica* 48 reported in that work as BSP14, was more drought tolerant than the *F. virginiana* accession NCC85-13V. This conclusion was based on the ability to remain turgid at increasing soil water deficits and evidence of osmotic adjustment. Interestingly, *F. chiloensis* ssp. *pacifica* 48 exhibited the lowest I_d , g_T , and g_T ratio values for *F. chiloensis* in this study (Fig. 5). In contrast, the drought susceptible *F. virginiana* NCC85-13V exhibited some of the highest values within *F. virginiana*. Although it is not clear how much membrane competence may contribute to drought tolerance, this evidence suggests that it may be an important component.

Based on I_d , g_T , and g_T ratio values at 2 h for single accessions of the *Fragaria* species, *F. chiloensis* ssp. *lucida* 34 exhibited more membrane injury as RWC declined than the accessions representing the other species (Figs. 2–4). However, within *F. chiloensis*, a range of values were evident such that accession 34 exhibited greater membrane competence than accessions 24 and 135. A range of values was also evident in *F. virginiana*. Species means indicated the *F. virginiana* exhibited more injury to 50% RWC than *F. chiloensis*, conforming to conclusions from earlier studies (Zhang and Archbold, 1993a, 1993b) that *F. chiloensis* is more drought tolerant. However, due to the range in intraspecific and interspecific variation in these results, it is evident that not all accessions of a species share similar drought tolerance traits. Furthermore, sources of drought tolerance may be available from accessions of other *Fragaria* species (Figs. 2–4).

The I_d , g_T , and g_T ratio values for *F. chiloensis* 34 were similar in both experiments, while the values for *F. virginiana* 67 were greater in Expt. 1 than in Expt. 2. The reason for the variation by *F. virginiana* 67 between experiments is not known, but an examination of values for individual replications in Expt. 1 revealed two with values similar to those in Expt. 2. While this emphasizes the need for adequate replication in studies such as these, this problem might be encountered in cultivar improvement programs with limited numbers of plants, perhaps only one, of each advanced selection.

The slopes of the regressions of g_T and the g_T ratio versus RWC were better indicators of membrane competence in the species accessions than g_T values at a single RWC. Natural variation in ion leakage and resulting g_T values could mask dehydration stress effects and lead to erroneous conclusions. For example, although *F. virginiana* ssp. *glauca* 281 exhibited the highest g_T values at

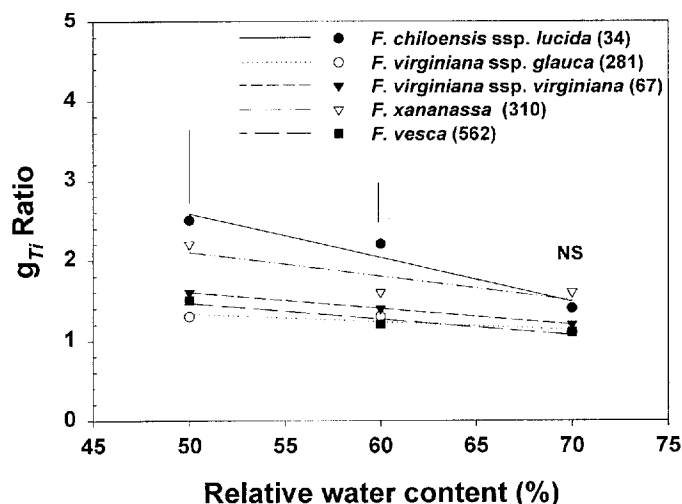


Fig. 4. Tissue ionic conductance (g_{Ti}) ratio, or g_{Ti} at the dehydration RWC to g_{Ti} at 100% RWC, of five *Fragaria* accessions at 2 h after leaf disk dehydration versus leaf disk relative water content (RWC). Each data point represents the mean of two experiments, three leaf disks per experiment. Vertical bars represent the least significant difference at $P = 0.05$. The regression equations are *F. chiloensis* ssp. *lucida* 34, (solid line), g_{Ti} ratio = $5.33 - 0.06RWC$, $r = 0.94$; *F. virginiana* ssp. *glauca* 281, (dotted line), g_{Ti} ratio = $1.84 - 0.01RWC$, $r = 0.75$; *F. virginiana* ssp. *virginiana* 67, (short dashed line), $Y = 2.63 - 0.02RWC$, $r = 0.99$; *F. xananassa* 310, (dash-dot-dot line), g_{Ti} ratio = $3.62 - 0.03RWC$, $r = 0.75$; and *F. vesca* 562, (long dashed line), g_{Ti} ratio = $2.47 - 0.02RWC$, $r = 0.92$. All regressions are statistically significant at $P \leq 0.05$. Accession numbers are in parentheses.

each dehydration level as well as at 100% RWC (data not shown), the increase in g_{Ti} was less across RWC levels than that for *F. chiloensis* 34 (Fig. 3). This suggests that *F. virginiana* ssp. *glauca* 281 has greater membrane competence in response to dehydration stress than *F. chiloensis* 34. Whether this means that *F. virginiana* ssp. *glauca* 281 is also more drought tolerant is less certain until appropriate whole-plant studies are performed. A leakier membrane in nonstressed leaves of *F. virginiana* ssp. *glauca* 281, indicated by its g_{Ti} values, might not be adequately tight to function appropriately or maintain turgor as water stress increases. The leaves of *F. virginiana* ssp. *glauca* 281 appear to lack turgidity even when well-watered (personal observation).

Because the derivation of g_{Ti} has a more clearly defined theoretical basis, describing an actual rate of ion loss from leaf disks, it would seem preferable to using I_d . Since tissue EC concentration was similar among accessions, and as a result the relative concentration gradient between the disks and the incubation solutions was also similar among samples, leaf thickness as an estimate of cut surface area was the primary difference among accessions. Calculating g_{Ti} eliminated leaf thickness as a variable, so rates of ion loss may accurately reflect stress effects on foliar membranes. Using the regression of g_{Ti} and g_{Ti} ratio across RWCs, the relative response of *F. chiloensis* to dehydration stress was greater than the other species. The I_d values also indicated this. While I_d or some variant has been used to characterize drought tolerance in some studies (Martin et al., 1987; Premachandra et al., 1991, 1992; Vasquez-Tello et al., 1990), others have indicated that I_d values are not reliable indicators of freezing or drought tolerance (Murray et al., 1989; Whitlow et al., 1992). The results presented in this paper indicate that g_{Ti} , g_{Ti} ratio, and I_d , when regressed against RWC, each identify a *F. chiloensis* accession as having less membrane competence than the other species. Both g_{Ti} and I_d characterized relative freezing responses similarly in cabbage (*Brassica oleracea* L.) (Manley and Hummel, 1996). Due to the need to measure leaf thickness for g_{Ti} , I_d was easier to perform and seems an acceptable

technique especially if shorter incubation times (i.e., 1 to 4 h) are used. Significant differences in this work were only evident after 2 and 4 h.

Membrane repair may have occurred after several hours when g_{Ti} values were lower than at early sampling times. The decline in g_{Ti} values over time was also reported by Whitlow et al. (1992). Leopold et al. (1981) reported that leaf disks of cowpea desiccated to 69% initial fresh mass reached equilibrium with the bathing solution 2 h later than nondesiccated disks. The time difference was believed due to the time required for repair of the membranes. Different species or cultivars within species might not only exhibit differing levels of membrane competence in response to dehydration stress, but they may also exhibit varying capacities and rates of membrane repair. Measurements taken within the first few hours after dehydration stress may be the most accurate representation of injury not confounded by rates of repair.

The g_{Ti} value represents a conductance across a surface, but it is not without its limitations as Whitlow et al. (1992) discussed. While it purports to represent a conductance, it is not specifically that of a cell membrane even though it is being used to describe the effect of drought stress on membrane competence. Rather, it assumes conductance across the cut surface at the margin of the leaf disk which is not uniformly or continuously membrane bound. In addition, the cut surface encompasses a cylinder and is not planar, so diffusion is radial instead of in one dimension. Fick's First Law describes conductance across a planar surface with a concentration gradient in one axis such as a leaf surface. If g_{Ti} is to be used to examine specific rates rather than general differences among treatments, the equation may need modification to account for radial diffusion as discussed by Crank (1975). However, theoretical considerations aside, g_{Ti} may be a reliable approximation of ionic conductance across the cut surface and may be a useful method for estimating rates of ion loss in response to dehydration or other stresses.

Table 2. Mean leaf thickness, total solution EC after freezing and thawing, leaf EC concentration, and relative water content (RWC) after desiccation of *F. chiloensis* and *F. virginiana* accessions.

Species accession	Leaf thickness ^a (mm)	Total EC ^b (μS)	EC concn (μS·g ⁻¹ H ₂ O)	RWC
<i>F. chiloensis</i>				
CFRA 48	0.59 a ^x	49 b	3123 c	52 ± 1 ^w
CFRA 34	0.56 ab	65 a	3714 a	50 ± 1
CFRA 24	0.51 bc	47 b	3556 b	49 ± 3
CFRA 135	0.50 c	40 c	2834 d	46 ± 1
Mean	0.54	50	3307	50 ± 3
<i>F. virginiana</i>				
NCC85-13V	0.42 d	31 d	3112 c	44 ± 2
CFRA 67	0.33 e	32 d	3456 b	51 ± 1
CFRA 98	0.37 e	37 c	3337 bc	48 ± 1
CFRA 281	0.32 e	28 d	2858 d	48 ± 2
Mean	0.36	32	3191	48 ± 3
F Significance for species	*	*	NS	

^aLeaf thickness, $n = 10$.

^bTotal EC after freezing and thawing and EC concentration, $n = 6$. For EC concentration, fresh disk mass minus dry disk mass = g H₂O.

^xAcross all accessions, means followed by different letters are significantly different by LSD at $P = 0.05$.

^wRWC, $n = 3$, mean ± standard error of the mean.

^{NS}*Nonsignificant or significant F value for species means at $P \leq 0.05$.

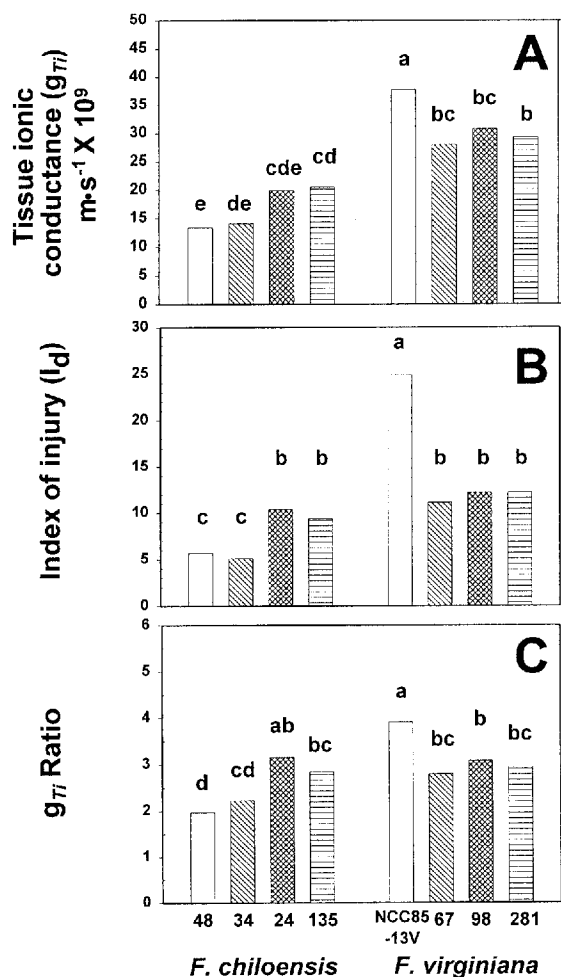


Fig. 5. (A) Tissue ionic conductance (g_{Ti}), (B) index of injury (I_d), and (C) g_{Ti} ratio of accessions within *Fragaria chiloensis* and *F. virginiana* at 2 h after leaf disk dehydration to 50% relative water content (RWC). Each bar represents the mean of three leaf disks. Bars with a different letter above are significantly different by least significant difference at $P = 0.05$.

In conclusion, there was significant variation in membrane competence in response to dehydration stress both within and among *Fragaria* species in the first few hours after stress was applied. Membrane competence may contribute to drought tolerance in *Fragaria* as two accessions exhibited responses conforming to their relative drought tolerance documented in earlier studies. While g_{Ti} is a useful expression for estimating the rate of ion loss from excised leaf disks, it was not clearly superior to I_d for identifying greater membrane competence. If g_{Ti} is used, control (100% RWC) values should also be determined to estimate a g_{Ti} ratio, i.e., treatment versus control. If both control and stress treatment EC values were available, I_d values could also be estimated for the samples. For screening large populations of advanced selections of *Fragaria*, I_d with short (1 to 4 h) incubation times may be adequate.

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