Transpiration decline curves and stomatal characteristics of faba bean genotypes

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Abstract

Nine F_1 crosses of faba bean (*Vicia faba* L.) and their parental genotypes were evaluated for transpiration decline curves (TDC) and stomatal characteristics. The most common type of TDC had one inflexion. One parent and three crosses were characterized by TDC of two inflexions, while one cross had TDC without inflexion. The genotypes which exhibited TDC with 2 inflexions showed later time to stomatal closure (SC). Also stomatal and cuticular transpiration rates differ significanlty among studied genotypes. A model for selecting genotypes with effective water retaining features was suggested. This model includes relative water content (RWC) at SC, stomatal density, guard cell length, stomatal pore width at both sides of the leaf, stomatal pore length at lower side and leaflet dry mass

Additional key words: crosses, cuticular and stomatal transpiration, drought tolerance, *Vicia faba*, water retaining features.

Introduction

Faba bean is drought sensitive and highly responsive to irrigation (Day and Legg 1983). Nerkar *et al.* (1981) and Tanzarella *et al.* (1984) reported significant genotypic differences of faba bean in the frequency and size of stomata. Therefore, differences of stomatal behaviour could be used in improving faba beans to achieve drought tolerance by reducing water loss and increasing the assimilation ratio. One method to determine stomatal behavior is to monitor the rate of water loss from detached leaves under known or constant environmental conditions (*e.g.* Quisenberry *et al.* 1982).

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Abbreviations: CT - cuticular transpiration; ESC - end of stomatal closure; GCL - guard cell length; GCW - guard cell width; RH - relative humidity; RWC - relative water content; SC - stomatal closure; SLM - specific leaf mass; SPL - stomatal pore length; SPW - stomatal pore width; SSC - start of stomatal closure; ST - stomatal transpiration; TDC - transpiration decline curves; WL - water loss.

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Roark *et al.* (1975) divided detached leaf drying curves (transpiration decline curves) into stomatal and cuticular phases and estimated the time and relative water content at stomatal closure. By automation of the mass determination and by direct computer analysis of the results this technique could be suitable for the evaluation of quite large number of genetic material (Jones 1979).

The objectives of this study were: *1*) to identify the variability among several faba bean genotypes and their crosses with regard to their transpiration decline curves (TDC), *2*) to determine the stomatal behaviour and the relative water content at stomatal closure and *3*) to determine whether these parameters are effective criteria to select cultivars for drought tolerance.

Materials and methods

Plants: Nine F_1 crosses of faba bean (*Vicia faba* L.) and their parental genotypes were grown during the winter season of 1994/1995 at the Agricultural Experimental and Research Center, Faculty of Agriculture, University of Cairo, Giza. Cultural practices were applied according to recommendations.

Transpiration decline curves (TDC): Detached leaves from plants in the flowering stage (85 d after sowing) were used to evaluate the rate and amount of water loss according to Quisenberry *et al.* (1982). From five plants of each genotype, the single uppermost fully expanded leaf was excised just prior to sunset (17.00 - 17.30). Immediately after excision, the leaf petiole was placed in water with its leaflets in air at 100 % relative humidity (RH). The leaves were allowed to rehydrate overnight in the dark. On the following day, the water saturated leaves were exposed to diffuse natural light for 90 min in air at 100 % RH to open their stomata. After this period, the leaflets were dried superficially with soft tissue paper and the cut surfaces of their petioles were immersed in melted paraffin (melting point 56 °C) to seal the conducting elements (Slavík 1974). Each leaf was left in a vertical position by the gentle fixation of its petiole in a narrow hole of a multiperforate plastic rack. Then the leaves were left in a diffuse natural light in the laboratory at 21 ± 0.9 °C and 58.8 % RH (range 49 - 67 % during different runs). The rate of water loss was determined for each leaf by weighing it on an electronic balance at 5 min intervals over 2-h period. At the end of the experiment, the leaflets were detached from the main axis of the leaf for the determination of their area and their dry masses (oven drying at 70 °C for 48 h). The obtained data were used to calculate the relative water content [RWC = (fresh mass - oven dry mass)/(saturated mass - oven dry mass) × 100] and the rate of water loss (WL). The relationship between RWC and time was distinguished into linear slope(s) which ranged from one to three. Analysis of regression and *t*-test of differences between regression coefficients were performed to identify the validity of distinguishing the decline curve into the slope(s). The TDC were distinguished into the following types: *1*) Curve with a single inflexion and two linear slopes. The first slope exhibited the rate of total mostly stomatal transpiration (ST) and the second one represented the water loss through cuticle alone (CT) and their intercept shows stomatal closure time (SC). *2)* Curve with two inflexions and three linear slopes indicating that SC occurs in two distinct phases; the first represents partial closure of stomata (SSC), while the second represents the end of stomatal closure (ESC). *3)* Curve without inflexion, *i.e.* where the analysis of regression and *t*-test were not statistically significant.

The area of leaflets was determined by the leaf area meter. The specific leaf mass (SLM) was calculated from the ratio: leaflet dry mass/ leaflet area.

Heterogeneity of regression coefficients for the group of genotypes, *i.e.* parents, crosses and both was performed to explore the significance of differences among the rates of ST and those of CT for each group according to Gomez and Gomez (1983). Also, this test was applied for each cross and its corresponding parents.

Stomatal characteristics were determined on the lowermost leaflet of the single terminal fully expanded leaves comparable to those used for TDC (five plants of each genotype). Optical micrometers (linear and square) were used to measure the densities of stomata (number per mm^2), the length (GCL) and width (GCW) of guard cells as well as the length (SPL) and width (SPW) of stomatal pore in epidermal strips obtained from the adaxial and abaxial surfaces of each leaflet by pulling off the epidermis. The epidermal strips were mounted in absolute ethanol and examined. The SPL and SPW were measured from epidermal strips using leaflets sampled at 10.00 local time (sunrise was at 5.50). Nerkar *et al.* (1981) indicated that the daily maximum leaf conductance in faba beans occurred 4 h after the start of the light period.

Results and discussion

Transpiration Decline Curves (TDC): Three types of TDC according to the number of inflexions were detected (Fig.1). All parents fitted to TDC with a single inflexion except L.536 while 3 crosses had TDC with 2 inflexions and one cross had TDC without inflexion. Comparision of the crosses with their corresponding parents indicated that 3 crosses exhibited one inflexion similar to both parents. Although the L.536 is a common female parent in four crosses, its F_1 (with other 4 parents) plants showed different types of TDC. The crosses L.536 × G.461 and L.536 × L.241 showed a TDC with a single inflexion, while L.536 × G.674 exhibited a curve with two inflexions, and the L.536 × G.402 had a TDC without inflexion which is the only exceptional case in the present study. Although the majority of parents had TDC with one inflexion, the F_1 crosses (G.461 × G.3 and L.101 × TW) exhibited the TDC which had two inflexions. The number of inflexions in the curve was not necessarily an indication of RWC and/or the duration of the time for stomatal closure. For all sets, except G.3 × R.B., R.B. × G. 402 and L.101 × TW, wide variations occurred among each cross and its parents in RWC at the SC. The change in RWC from the start to the end of SC was higher in cross G.461 × G.3 than in the crosses L.536 × G.674 and L.101 × T.W. In the parent L.536, the RWC at the end of SC (second inflexion) was 67.2 %, *i.e.*, 14.8 % lower than that at SSC. Three crosses behaved similarly, *i.e.* one of them included the parent L.536 as a female while the other crosses (G.461 × G.3 and L.101 × TW) did not follow the above mentioned performance (the two inflexion points). The decline of RWC during the period of stomatal closure has been noted by many authors (*e.g.* Pisek and Winkler 1953, Slavík 1958, Kramer 1983, El-Tantawy *et al.* 1990).

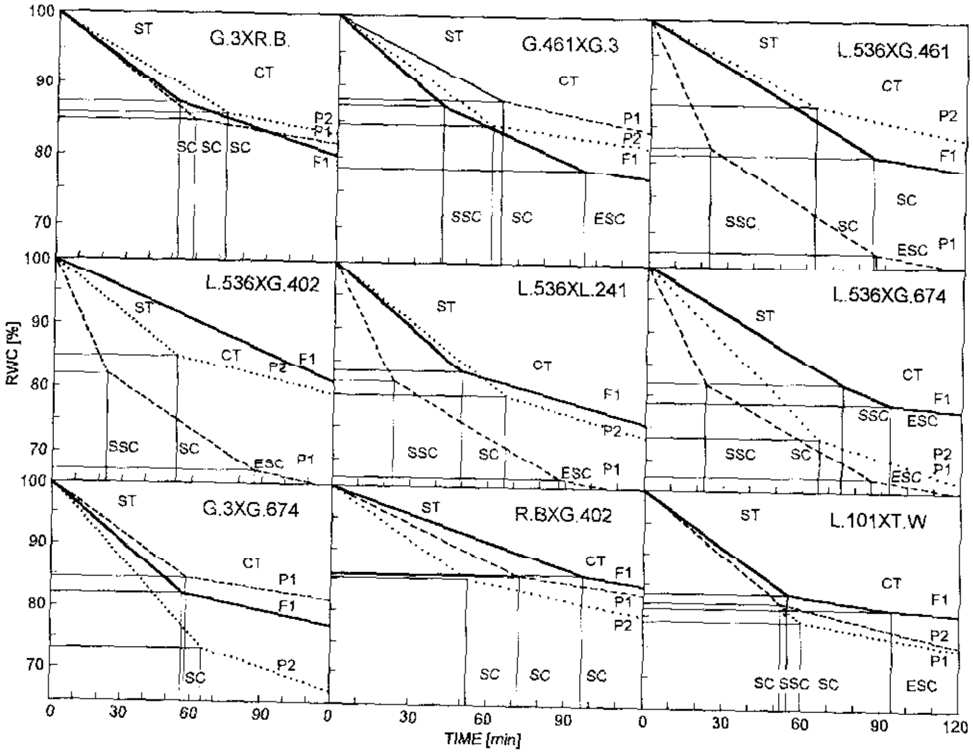

Fig. 1. Transpiration decline curves of faba bean crosses and their parents (CT - cuticular transpiration; ESC - end of stomatal closure; F_1 - F_1 cross; P_1 and P_2 - parents; RWC - relative water content, SC - stomatal closure; SSC - start of stomatal closure; ST - stomatal transpiration).

It is suggested that the parent L.536 had a lower degree of drought tolerance than the other crosses which had TDC with two inflexions. This is primarily attributed to the low RWC (67.2 %) at the end of SC in comparison to the F_1 crosses which had higher RWC at the end of SC. Okali (1971) and Nazrul-Islam (1983) suggested that species with low degree of drought tolerance have high stomatal sensitivity as an adaptation against the development of damaging water deficit.

Generally, it is worth to mention that all crosses and parents with TDC of two inflexions showed later time to stomatal closure than the parents and/or the other F_1 crosses which had TDC with a single inflexion.

Heterogeneity were significant among parents, crosses and all genotypes for both rate of ST and CT (Table 1). Rates of ST and CT of each cross significantly varied from those of its parents.

Table 1. Stomatal and cuticular transpiration rates [mg m^{-2} s^{-1}] of faba bean genotypes and their crosses (mean ± S.E.)

Genotype	Stomatal transpiration (ST)	Cuticular transpiration (CT)
G.3	13.7 ± 3.8	3.3 ± 0.4
R.B	10.2 ± 2.4	2.7 ± 0.3
G.461	8.8 ± 3.0	3.0 ± 0.4
L. 536	48.2 ± 5.1	4.5 ± 0.5
G. 674	18.0 ± 4.3	6.2 ± 0.5
G. 402	13.8 ± 2.8	3.7 ± 0.3
L. 241	19.5 ± 3.2	6.8 ± 0.4
L. 101	12.2 ± 2.1	4.0 ± 0.2
T.W	16.7 ± 1.8	3.0 ± 0.2
G.3 × R.B	9.2 ± 1.6	4.7 ± 0.3
G.461 × G.3	20.2 ± 2.1	2.8 ± 0.3
L.536 × G.461	11.2 ± 2.3	3.2 ± 0.2
G.3 × G.674	13.0 ± 1.6	3.2 ± 0.1
R.B × G.402	6.7 ± 2.0	3.3 ± 0.2
L.536 × G.402	8.8 ± 1.9	-
L.536 × L.241	18.0 ± 2.9	5.3 ± 0.2
L.536 × G.674	17.8 ± 2.4	3.5 ± 0.2
L.101 × T.W	12.8 ± 2.3	1.5 ± 0.1

Four crosses showed lower rates of CT than their parents and 3 ones exhibited intermediate rates of CT between their corresponding parents. Cross G.3 × R.B exceeded its parents with regard to the CT rate. Jones (1979) pointed out that cuticular conductance plays an important role under stress conditions when the stomata are closed.

Low CT could be a useful selection criterion to identify genotypes with enhanced ability to survive under severe water stress (Muchow and Sinclair 1989). Therefore, the faba bean crosses of the present investigation which posses low CT could be considered as a source of deriving lines characterized by low CT rates. The three crosses (G.3 × G.674, L.536 × L.241 and L.536 × G.674) showed higher ST rates than their corresponding parents, but lower CT than their parents (Table 1). The reverse holds true for G.3 × R.B (Fig. 1).

Model of water retaining features: Stomatal density and GCL at abaxial side of the leaf as well as SPW at upper side and leaflet dry mass (Table 2) positively influenced the RWC at SC, whereas, RWC at SC was negatively affected by stomatal density and GCL at upper side as well as SPL at lower one. This model explains 93 % of the variation in RWC at SC. Therefore, selection of genotypes having such traits could improve the level of RWC at SC which agrees with the findings of Nerkar *et al.* (1981). For the majority of parents and their F_1 crosses, there were fewer stomata and smaller guard cells (length and width) on the adaxial surface (Table 2). Since amphistomaty and larger guard cells could result in higher stomatal conductance and consequently higher transpiration and photosynthetic rates (Mott *et al.* 1982), it is

Table 2. RWC [%] at stomatal closure, stomatal density [No. mm^{-2}] and size [µm] and leaflet dry mass [mg] as well as model indices ($I = \Sigma b_i x_i$ where b_i is the coefficient and x_i is the measured value of the features according to multiple regression model) of studied faba bean crosses and their parents. Mean ± S.E.

Genotype	RWC at SC	Density lower	upper	GCL lower	upper	SPL lower	SPW lower	upper	Dry mass	I
G.3	84.5	58.3 ± 6.7	42.1 ± 5.8	47.0 ± 1.1	41.0 ± 4.2	34.0 ± 1.4	2.8 ± 1.4	3.3 ± 0.7	45.8	20.6
R.B	85.5	53.3 ± 4.0	49.4 ± 3.2	49.5 ± 1.1	47.0 ± 2.1	32.0 ± 5.7	3.0 ± 0.0	4.5 ± 0.7	72.7	21.4
G.461	87.8	55.5 ± 6.3	29.9 ± 1.6	46.5 ± 2.2	44.5 ± 1.1	30.5 ± 2.1	6.5 ± 1.3	5.8 ± 1.7	60.5	21.4
L. 536	67.2	37.2 ± 2.7	42.5 ± 3.1	48.5 ± 2.2	47.0 ± 3.3	36.5 ± 2.5	10.0 ± 1.8	6.5 ± 2.1	78.9	3.1
G. 674	73.5	44.4 ± 1.9	54.7 ± 5.5	44.5 ± 3.3	44.5 ± 2.1	30.5 ± 3.3	7.0 ± 1.1	8.5 ± 1.4	52.9	7.9
G. 402	85.0	54.7 ± 1.8	45.1 ± 3.6	51.8 ± 1.7	49.3 ± 4.5	35.5 ± 2.7	7.0 ± 1.1	9.5 ± 2.1	53.2	20.2
L. 241	79.9	58.7 ± 4.7	50.1 ± 1.6	47.4 ± 2.1	45.5 ± 2.1	30.0 ± 2.0	5.3 ± 1.6	6.8 ± 1.1	40.7	17.9
L. 101	82.0	43.6 ± 2.5	38.9 ± 2.8	51.5 ± 1.4	46.0 ± 2.2	34.3 ± 2.4	7.5 ± 2.5	4.5 ± 1.4	54.7	15.5
T.W	79.0	49.1 ± 4.9	29.1 ± 1.6	48.5 ± 1.4	48.0 ± 2.1	31.5 ± 2.9	6.5 ± 2.2	5.5 ± 1.9	70.4	16.3
G.3 × R.B	86.4	52.5 ± 2.0	44.2 ± 2.8	46.0 ± 2.9	44.5 ± 2.1	28.0 ± 3.3	4.0 ± 0.6	3.8 ± 0.9	72.1	20.4
G.461 × G.3	78.0	67.8 ± 2.0	44.8 ± 1.4	45.0 ± 1.8	45.0 ± 1.8	28.3 ± 1.7	5.5 ± 1.9	3.5 ± 1.1	47.2	11.8
L.536 × G.461	81.0	46.3 ± 4.2	36.6 ± 1.5	47.5 ± 2.5	47.5 ± 1.8	33.3 ± 1.7	4.5 ± 2.1	7.0 ± 3.3	67.3	14.1
G.3 × G.674	82.0	60.5 ± 6.9	53.0 ± 6.0	43.5 ± 2.2	41.5 ± 1.6	31.5 ± 3.4	4.8 ± 0.6	3.5 ± 1.1	91.8	18.7
R.B × G.402	85.8	78.6 ± 5.9	57.1 ± 4.2	47.5 ± 2.5	44.5 ± 2.7	33.0 ± 2.1	3.8 ± 1.3	3.5 ± 1.4	58.4	20.5
L.536 × G.402	81.2*	47.0 ± 3.2	34.5 ± 2.5	46.5 ± 5.5	43.0 ± 2.1	29.5 ± 2.1	7.3 ± 2.2	5.5 ± 1.9	63.5	21.5
L.536 × L.241	89.2	59.1 ± 3.2	61.9 ± 8.8	46.5 ± 2.2	43.0 ± 2.7	33.0 ± 3.7	4.0 ± 1.4	5.0 ± 2.5	85.4	22.8
L.536 × G.674	79.0	37.0 ± 3.6	37.2 ± 7.7	46.5 ± 3.4	44.5 ± 1.1	29.5 ± 5.7	2.8 ± 2.1	3.3 ± 1.7	65.9	11.5
L.101 × T.W	81.0	58.8 ± 5.1	43.1 ± 2.5	43.0 ± 6.5	45.0 ± 4.0	27.0 ± 4.1	6.0 ± 1.4	6.5 ± 1.1	71.1	16.2

* - at the end of experiment without closure.

apparent that the occurrence of higher stomatal density on the abaxial leaflet side of the genotypes investigated would result in higher abaxial than adaxial stomatal conductance (Nobel 1991). Mott *et al.* (1982) indicate that the occurrence of fewer stomata on the adaxial surface is a general feature of plants growing in sunny habitats. This pattern of stomatal density was observed in the majority of the studied parents (except G.674) and in F_1 crosses (Table 2). This feature is already recorded in faba bean by Kassam (1973). Data of comparing the indices (I) for all genotypes with RWC at SC and the averages of these features (Table 2) proved that this index is highly correlated with RWC at SC.

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