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WATER STRESS TOLERANCE AND PROLINE ACCUMULATION IN PHASEOLUS VULGARIS L.

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SUMMARY

The water stress tolerance of five agronomic varieties of *Phaseolus vulgaris* of different geographical origin in Tanzania was studied. As indicators of water stress tolerance water saturation deficit (WSD), stomatal resistance, and flower and pod abscission were used. Of three of these varieties also proline accumulation under stress conditions was determined. In both respects the varieties showed significant differences. Between water stress tolerance and proline accumulation under stress conditions a positive correlation was found.

The possibility to use proline accumulation as an indicator in selection schemes for water stress tolerance in *Phaseolus vulgaris* is discussed.

1. INTRODUCTION

YANCEY et al. (1982) gave evidence for the existence of strong selective pressure in organisms under some form of water stress. Under these conditions the ratio of osmolytes to water and to macromolecules in the cell changes. Among the osmolytes changing in concentration YANCEY et al. mention in particular glycine, alanine, proline, taurine, and β-alanine.

Barley varieties which accumulated larger concentrations of proline were found to survive more extreme stress conditions than those showing lower proline levels (SINGH et al. 1973). In contrast, HANSON et al. (1977) and ILAHI & DÖRFFLING (1982) found a negative correlation between proline accumulation and water stress tolerance in barley, and in maize.

In view of this apparent controversy the present study was undertaken to elucidate a possible correlation between water stress tolerance and proline accumulation.

2. MATERIAL AND METHODS

In this study five agronomic varieties of *Phaseolus vulgaris L.* from Tanzania were used:

Burundi Njano Fupi	BNF
Ulyankulu Mpanda Kitenge	UMK
Bukoba Goroli Kahawia	BGK
Kigoma Njano Ndefu	KND
Ulyankulu Kitenge Nyeusi	UKN

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The seeds were germinated in vermiculite. After the seedlings had entered the four leaf stage they were transplanted in pots (Ø 14 cm) with garden soil and watered normally. The plants were grown in a climate chamber under short day conditions (8L/16D) at 25 ± 1 °C, c. 60% relative humidity, and illuminated with fluorescent tubes (Sylvania 215 W, VHO) supplemented with incandescent lamps, producing c. 10,000 lux at the plant level. After two months water stress was initiated by withholding water during either two or five days, following by one watering with a fixed volume of water. After this regime was maintained for one month, water saturation deficit (WSD), stomatal resistance, and flower and pod abscission were assessed. WSD was estimated using STOCKER's (1929) techniques as modified by KAPUYA (1975); stomatal resistance was measured with the xylene and nujol infiltration technique of ALVIM & HAVIS (1954); flower and pod abscission figures were obtained by expressing the number of abscised flowers or pods as percentage of the total number produced.

Based on the results of these experiments the three varieties BGK, KND and UKN were selected to study proline accumulation. During eigth weeks plants of these varieties were grown in water culture, in Hoagland solution. Then polyethylene glycol 400 (PEG 400) was added to the culture medium to produce water potentials of −2.5, −5.0, −7.5, and −10.0 bars. The control plants received no PEG 400.

After ten days of PEG treatment stomatal resistance, relative water content, and proline level were determined. For proline determination uniformly positioned leaves from each treatment were harvested and 200 mg portions were used for amino acid extraction (LINSKENS & TUPÝ 1966) and analysis (with a JEOL-64

Table 1. Water saturation deficit in five varieties of *Phaseolus vulgaris* in response to water stress. Results are expressed as mean percentage values ± SE for five replicates.

Treatment	Varieties				
	BGK	BNF	KND	UMK	UNKN
Control	21.0 ± 2.4	24.0 ± 1.8	17.0 ± 1.7	19.5 ± 2.1	14.0 ± 1.9
2 d interval	26.5 ± 3.1	31.3 ± 2.2	35.5 ± 2.9	30.6 ± 3.4	26.0 ± 1.8
5 d interval	37.0 ± 2.3	56.0 ± 1.2	61.2 ± 2.0	40.2 ± 3.7	53.7 ± 1.5

Table 2. Relative stomatal opening (reciprocal stomatal resistance) in five varieties of *Phaseolus vulgaris* in response to water stress as measured by the xylene/nujol infiltration techniques. Results are expressed as mean serie values of xylol/nujol infiltrating mixtures ± SE for five replicates.

Treatment	Varieties				
	BGK	BNF	KND	UMK	UKN
Control	7.0 ± 1.3	6.8 ± 1.1	9.3 ± 1.0	10.7 ± 1.5	7.8 ± 0.6
2 d interval	1.5 ± 0.6	5.0 ± 0.7	1.8 ± 0.5	5.8 ± 0.9	6.8 ± 0.3
5 d interval	1.6 ± 0.8	6.5 ± 1.2	2.2 ± 0.4	4.3 ± 0.5	7.1 ± 0.9

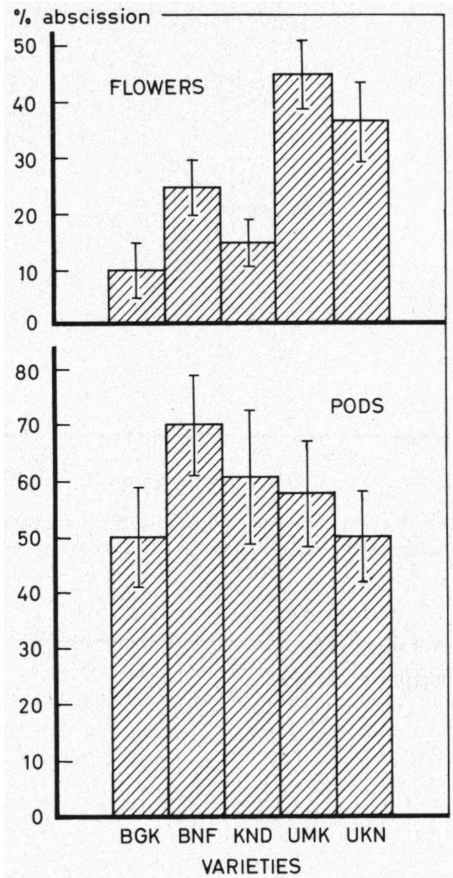

Fig. 1. Abscission of flowers and pods in five varieties of *Phaseolus vulgaris* Percentage of total numbers of flowers formed.

AH analyser). The retention time, as indicated by standard amino acids, was used to identify the individual amino acids.

3. RESULTS

Table 1 shows an increase in WSD under increasing water stress conditions in all *Phaseolus vulgaris* varieties, the largest in var. KND, the lowest in BGK and UMK.

Under the same stress conditions the relative stomatal opening (*table 2*) is little affected in the varieties BNF and UKN, but in the varieties BGK, KND, and UMK decreases considerably and stomatal resistance increases correspondingly.

Fig. 1 demonstrates that varieties BGK and KND have the lowest, UMK and UKN the highest rate of flower abscission, BNF the highest, BGK and

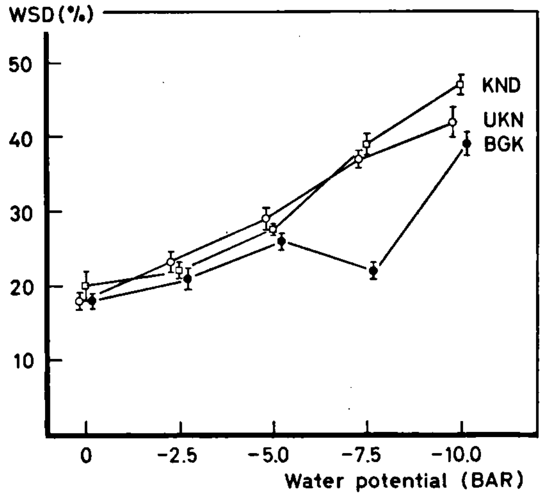

Fig. 2. Water saturation deficits (WSD) of three *Phaseolus vulgaris* varieties under increased water stress conditions, i.e. decreasing water potentials obtained with polyethylene glycol in hydroculture.

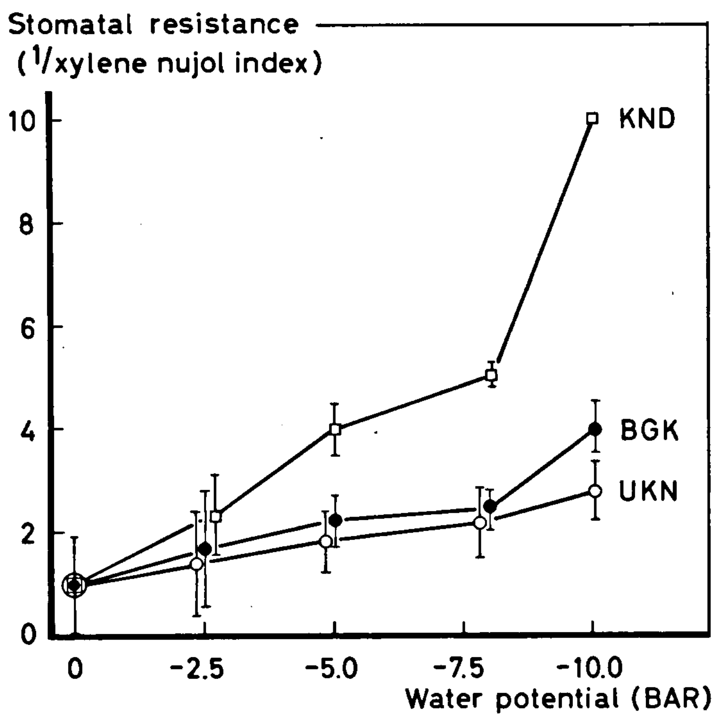

Fig. 3. Stomatal resistance in three *Phaseolus vulgaris* varieties under increased water stress. Same conditions as in *fig. 2*.

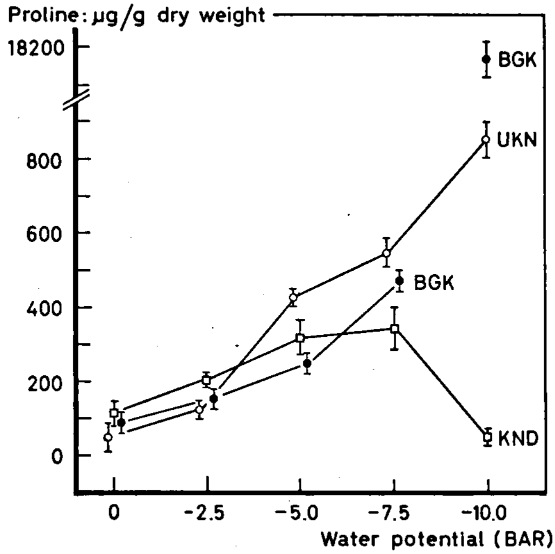

Fig. 4. Proline accumulation in three *Phaseolus vulgaris* varieties under increased water stress; same conditions as in *fig. 2.*

UKN the lowest pod abscission. Apparently there is no correlation between both phenomena.

Fig. 2 shows that under increasing water stress conditions obtained by polyethylene glycol WSD of all three selected varieties BGK, KND and UKN increases more or less to the same extent, whereas *fig. 3* demonstrates a much stronger increase in stomatal resistance with increasing water stress in variety KND than in the other two.

Fig. 4 shows that with increasing water stress up to a water potential of –5 bar proline accumulation, on the basis of dry weight, takes place at more or less the same rate in all three varieties. A further decrease in water potential from –5 to –10 bar results in a particular strong proline accumulation in variety BGK, a much smaller increase in variety UKN, but a drop in proline content in variety KND.

Similarly, under increasing water stress up to a water potential of –7.5 bar (*fig. 5*) the proline percentage of the total amino acids remains more or less constant. However, a further decrease of the water potential to –10.0 bar results in a steep rise of the proline percentage of the total amino acids in variety BGK, while in the other two varieties the proline percentage remains constant.

4. DISCUSSION

Changes in parameters like WSD, stomatal resistance, flower and pod abscission as reported here are indicative of adverse growth and development of the plant. The results presented in *table 1* and *2* and *figs. 1–5*, and those summarized in

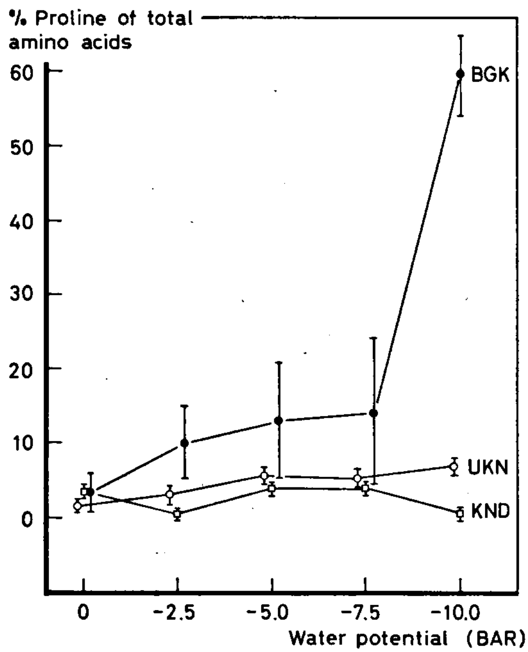

Fig. 5. Proline per cent of total amino acids in three *Phaseolus vulgaris* varieties under increased water stress: same conditions as in *fig. 2*.

Table 3. Summary of part of the exerimental data. Highest values in **bold** type, lowest values in *italics*.

Variety	BGK	BNF	KND	UMK	UKN
Increase in stomatal resistance, "5 day interval" minus control	**4.8**	*0.1*	3.5	1.4	*0.1*
WSD, "5 day interval", % of control	*176*	233	**360**	206	**384**
minus control	*16,0*	32.0	**44.2**	20.7	**39.7**
Flower abscission, "5 day interval", %	*10*	24	15	**44**	36
Pod abscission, "5 day interval", %	*50*	**70**	60	58	*50*
Stomatal resistance, –7,5 bar	*2.5*	–	**5.0**	–	*2.2*
–10.0 bar	4.0	–	**10.0**	–	3.0
WSD, % of control, –7.5 bar	*122*	–	**217**	–	206
–10.0 bar	*217*	–	**235**	–	**233**
Proline, µg/g, minus control –7.5 bar	380	–	*220*	–	**500**
–10.0 bar	**18110**	–	*–70*	–	835
Proline, % of total amino acids –7.5 bar	**14**	–	*4*	–	5
–10.0 bar	**60**	–	*1*	–	7

table 3 demonstrate that within the species *Phaseolus vulgaris* significant varietal differences exist in the reaction to water stress. Variety BGK is the most water stress tolerant: when stress is imposed a pronounced increase in stomatal resistance results, little water is lost as indicated by a small change in WSD, and flower and pod abscission are the lowest of the five varieties. On the other hand variety KND is the least tolerant to water stress: upon water stress the stomatal resistance increases considerably, nevertheless it develops the highest increase in WSD and the rates of flower and of pod abscission are rather high. The plants seem to dry and die much earlier than those of variety BGK. In variety UKN upon water stress the stomatal resistance hardly increases, the increase in WSD is high, flower abscission considerable, pod abscission low, and vegetatively it seems to survive best of the five varieties tested.

Under stress situations plants may exhibit adaptational changes. In adaptation to water stress proline is considered to be involved (STEWART & LEE 1974). Proline is one of the compatible osmolytes which will enhance cell wall retention while macromolecular functions remain unaffected (YANCEY et al. 1982). The accumulation of proline in plants under identical stress conditions has been shown to be species-specific (CAVALIERI & HUANG 1979), and also within a species, variety-specific (HANSON ET AL. 1977; ILAHI & DÖRFFLING 1982). However, whereas Ilahi & Dörffling found in four varieties of *Zea Mays* and Hanson et al. in two cultivars of barley a negative correlation between proline content under water stress and stress tolerance, in the present study a positive correlation is established. When the osmoregulatory role assigned to proline by STEWART & LEE (1974) is considered, the present findings would offer an explanation for the better survival of the varieties BGK and UKN as opposed to the premature desiccation and death of variety KND. It would appear that the high proline levels observed in variety BGK actually contribute to a better cell water retention. This fact is supported by the low WSD values in variety BGK. SCHOBERT (1979) has also suggested that high proline accumulation may assist in the protection of the enzymes of the plant by binding water to the proteins and thus maintaining their hydration. These results are in line with those of SINGH et al. (1973) who observed that barley varieties which accumulated larger amounts of proline tended to have leaves which survived extreme water stress more readily and grew more rapidly following stress relief.

Determination of the capacity of proline accumulation under stress conditions could be a useful index in plant breeding practices selecting for stress tolerant varieties within a species.

This is of particular importance to a country like Tanzania where such water stress tolerant varieties are in high demand because of the scarcity of the rains in the greater part of the country. It is doubly important when the variety involved is protein-rich, as with *Vicia faba*, because of the acute shortage of protein foods which increase the incidence of protein deficiency diseases in man.

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