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MEASUREMENT OF SOIL WATERLOGGING TOLERANCE IN *PHASEOLUS VULGARIS* L.: A COMPARISON OF SCREENING TECHNIQUES

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ABSTRACT

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Three methods not reported previously to determine flooding tolerance, and a
scoring system developed for this study, were utilized to evaluate the effects c
waterlogging upon 3 bean genotypes empirically differing in tolerance. These me
consisted of: (1) Triphenyl Tetrazolium Chloride Reduction Method (TTC), which
sures flooding tolerance by determining the rate of root respiration during stres
Electrical Conductivity Method (EC), in which the degree of root cell membrane int
is determined; and (3) Pressure Chamber Method (PC), by which the degree of
desiccation is found by measuring the water potential of the xylem. A visual s
method based on the external appearance of the plant was also used. The agree
among methods was generally good. The TTC and EC methods were more time-co
ing and results more variable, especially from the latter. Results of the PC and
scoring methods were highly correlated (r=0.85). However, TTC and EC require
7-day-old seedlings, thus allowing the screening of large numbers of samples an
efficient use of space. The F_1 and F_2 progeny from crosses between the most to
genotype (P074) and the most sensitive genotype (P149) indicated that differen
reaction to flooding are probably heritable.

Keywords: *Phaseolus vulgaris* L.; soil waterlogging tolerance.

ABBREVIATIONS

EC = Electrical Conductivity; PC = Pressure Chamber; TTC = Tripl
Tetrazolium Chloride Reduction.

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RODUCTION

'ommon bean (*Phaseolus vulgaris* L.) production in some parts of the ld is often limited by heavy rainfall, which frequently leads to water- ed conditions in poorly drained soils (CIAT, 1977). During waterlogging, bition of root respiration and the subsequent energy transfer to the root ie has been suggested as a cause of flooding stress (Rowe and Catlin, 1). Alternatively, toxic effects of the products of anaerobic respiration been suggested as the mechanism of root damage in both herbaceous tree species (Crawford, 1966, 1967; Crawford and McManmon, 1968; tts and Philipson, 1978).

ome of the effects of flooding resemble physiological drought (Kramer, 1; Regehr et al., 1975; Broue et al., 1976). Thus, one consequence of ding stress could be lowering of leaf water potential. A pressure chamber nique (Scholander et al., 1964) has been used intensively for rapid surement of leaf water potential.

oss of membrane integrity following flooding stress, resulting in leakage olutes, has been reported by Grineva (1962). A conductivity method cter et al., 1932) measuring ion efflux following stress injury has been successfully to evaluate relative freezing and heat-stress tolerance of to species (Li and Palta, 1978; Palta et al., 1981). Furthermore, ion ct following stress injury has been reported to be due to specific alter- ns in the membrane transport system (Palta and Li, 1980).

eduction of 2-3-5-triphenyl tetrazolium chloride, known as the TTC 10d (Kuhn and Jerchel, 1941; Kittock and Law, 1968), has been used to rmine relative heat and freezing stress resistance (Steponkus and Lan- ir, 1967; Towill and Mazur, 1975; Palta et al., 1981).

he existence of genetic variability for flooding tolerance is well known gers, 1974; Broue et al., 1976). Study of the genetic mechanisms asso- d with flooding tolerance has been the subject of several investigations res et al., 1977; Yoshida and Todano, 1978). Marshall and Millington 7) inter-crossed 5 clover cultivars varying in sensitivity to flooded soils, reported each cross to be more tolerant than either of the parents. ently, Hely and Zorin (1977) reported an increase in waterlogging ance in alfalfa by subjecting plant material to rigid selection.

e investigated the potential of using TTC, conductivity and pressure- nber techniques as rapid screening methods for measuring flooding ance.

ERIALS AND METHODS

t material. — Three bean (*Phaseolus vulgaris* L.) genotypes (P074, P254 P149), differing in response to soil waterlogging, were chosen from ng several lines that had been screened in preliminary studies following isition from the Centro Internacional Agricultura Tropical (CIAT).

From preliminary tests using visual scoring to determine tolerance, genotypes were classified as tolerant, sensitive and sensitive, respecti

TTC method. — Twenty-five seeds of each genotype were germinated grown inside a rolled moist paper towel. After 7 days, 3 seedlings from genotype were selected for homogeneity of primary root length.

The terminal 5 mm of each root were discarded, and three 1-cm segm were excised and weighed immediately. Three root segments from on the 3 seedlings within each line were then selected as control (non-stres samples and were immediately treated with TTC. The remaining 3 segm from each of 2 seedlings from each genotype were stressed to simu flooded conditions by submersion in 10 ml of dd water and placed in a room at room temperature (22° C). Specially prepared wide-mesh sc discs were inserted over the segments to ensure complete submersion fo total length of the treatment. Three segments of one seedling from genotype were thereafter removed successively at 12- and 24-h inte following immersion and treated with TTC. The dd water used had a 6 mg l^{-1} of dissolved oxygen, which was about 75% of the oxygen con tration of air. As root segments respire and use up the oxygen in water tissue experiences a progressively lower oxygen level.

The method for TTC treatment was similar to that of Palta et al. (19 Briefly, the root segments were transferred at a desired time to test t containing 3 ml of 0.08% TTC in 0.05 M phosphate buffer of pH 7.4. samples were incubated for 24 h in the dark at 22° C, the reduced TTC extracted, and absorbance was recorded at 485 mm with a Bausch Lomb 20 spectrophotometer. Data analysis was conducted according randomized complete-block design with 3 observations for each experi tal unit and the 3 time-periods considered as blocks.

EC method. — The procedure used was similar to that of Palta et al. (1 1978). The root-segment samples were prepared as for the TTC test. Im diately after excision, all segments were individually transferred into 3 vials, submerged in 10 ml of dd water, and the electrical conductivit the effusate in each vial recorded after the samples had been gently sh for 1 h. This reading was taken as the non-stressed reference level. vials thereafter remained undisturbed in a dark room at room tem ture (22° C). After 8, 12, 24 and 36-h intervals following the initiatio flooding, conductance was read to determine the amount of ion lea After the final measurement, following 36 h of root submersion, the containing 10 ml of solution and root segment, were quickly frozen to a −20° C and were then thawed by transfer to tap water at about 25° C. quick thaw procedure results in killing of the cells and releases all the into solution (Palta et al., 1981). The conductivity reading of the k tissue gave the total ion content of tissue. The amount of ion leakage d flooding was determined by dividing the 8-, 12-, 24- and 36-h reading

reading of the killed tissue. Data were analyzed as from a randomized nplete-block design.

method. — From each of the 3 genotypes, 96 uniformly-appearing seeds e planted in the greenhouse, 2 each in 12.5-cm diameter pots containing ilized sand. Plants were thinned to one per pot and watered twice daily ı a nutrient medium similar to Hoagland's solution (25 g $NH_4H_2PO_4$, 2 g KNO_3, 47.2 g $Ca(NO_3)_2$, 28.4 g $MgSO_4$ and 0.34 g trace elements ed to 132 l of tap water). Supplemental fluorescent lighting was supplied a schedule of 16 h at an intensity of 60 mc, 6 dm above the plant tops. temperature was maintained at 21/16°C day/night.

fter 65 days, during which all of the flower buds of the plants had been tinuously removed to allow more plant-to-plant developmental uniform- 2 plants from each of the 3 genotypes were removed from the green- se and placed in a growth chamber with lighting and temperature condi- ıs similar to that of the greenhouse.

'he flooding treatment was produced by inserting the pots containing the ıts into 16-cm diameter sealed plastic pots. Tap water was added to the er container and maintained at a level 5 cm below the surface of the sand ɔughout the experiment. After 72 h, each plant was removed from the nber, scored visually, and a pressure chamber was used to measure the em water potential of the 3 largest leaves (Boyer, 1967). For this pur- e, leaf sampling and preparation consisted of excising the most recently y expanded leaf and placing it in a small plastic bag to reduce loss of er during measurement. This experiment was repeated 4 times. Each e, 2 plants were taken from each genotype.

ı another experiment, the relationship between visual appearance and xylem water potential of 5 plants from each genotype was determined ng 72 h of stress. The visual evaluation of such external symptoms as and plant desiccation, discoloration, curling and collapse was accom- ıed by establishing a 1—6 stress rating. A rating of "1" indicated no ptoms, and each whole number thereafter represented a 20% increase. se data were then correlated on an individual plant basis with the xylem er potential measurement.

eritance studies. — The 3 genotypes were used as parents in preliminary eritance studies. F_1, F_2 and back-cross populations were generated and ted in the greenhouse. Cultural practice and environmental control e similar to that of the pressure-chamber experiment described above. andomized block design with 4 replications was used. Each block con- ed three 11.5 cm-diameter pots, representing each of the parental, F_1 reciprocal back-cross generations, and 8 pots of each of the 4 F_2 genera- s, i.e. including reciprocals.

looding treatment was applied in a similar way to the procedure de- ɔed under pressure-chamber experiment. The data from each generation within the 4 populations were tabulated to observe progeny distribu and segregation. The presence of significant phenotypic deviations from mid-parental value of the genotypes evaluated was tested by using the L Significant Difference.

RESULTS AND DISCUSSION

TTC method. — The TTC method was effective ($P \leqslant 0.05$) in differentia among the 3 genotypes, although no effect was perceived among the per of stress (Fig. 1). P074 demonstrated a very high TTC reducing ability, w P524 demonstrated a rapid decline during submersion for 24 h; P149 intermediate (Fig. 1). There was uniformity ($P > 0.05$) among the 3 sam within the same genotype and stress period. Except for P149, the differe in tolerance among the genotypes, based on the TTC method, agreed visual rating (Table I).

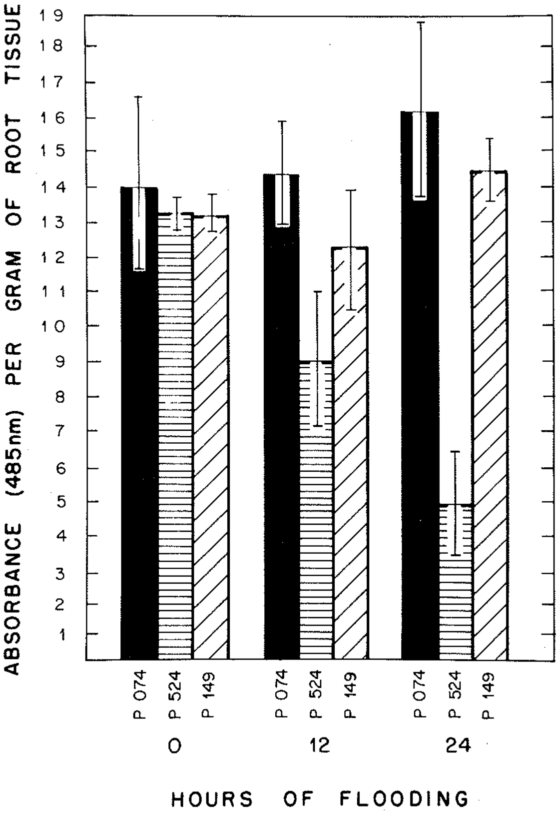

Fig. 1. The effect of flooding for 12 or 24 h as compared to no flooding (0 h) on the ity of bean seedling root-tip segments from genotypes P074, P524 and P149 to re TTC. Increasing ability to reduce TTC indicates increasing flooding tolerance. indicate the mean ± 1 standard deviation of 3 samples from each genotype within time sequence.

BLE I

nmary of the response of 3 bean genotypes to 4 methods of measuring flooding rance

otype	Method of measurement			
	TTC	EC	PC	Visual index
4	Tolerant	Tolerant	Tolerant	Tolerant
4	Sensitive	Sensitive	Semi-sensitive	Sensitive
9	Tolerant	Tolerant	Sensitive	Sensitive

'he flooding of soil decreases the supply of oxygen to roots, as compared normally-aerated soil, by a factor of 3×10^6 (Drew, 1979) and therefore ibits aerobic respiration of the living tissue. TTC reduction is thought to icate the status of oxidation—reduction reactions in the cell (Palta et al., 31). The measure of TTC reduction ability can thus be taken as indicative the rate of respiration in the tissue. It can be expected that during flood- of plants, the tolerance to waterlogging will be directly related to the ent of TTC reduction ability maintained by the tissue. A 50% loss in TTC uction ability has been found to be approximately related to the irrevers- injury produced by the freezing and heat stress in potato species (Palta l., 1981). It is possible that 24 h of flooding stress produced irreversible ry in P524 and no significant injury in P074 and P149.

method. — In general, the response trend of the genotypes to water- ging over the 36-h period was similar to that of the TTC experiment, with 24 susceptible and P074 and P149 tolerant, with a significant difference ween the 2 groups (Fig. 2). The length of time required for a plant tissue ple to lose 50% of its cell solutes to the surrounding environment is erally understood as the critical point of stress where the tissue sample nable to recover to a viable, functional condition (Palta et al., 1978). s stage was attained only by P524 after 8 h. P524 was also the only otype to reach a "non-viable" condition upon prolonged flooding (visual ervations). As in the TTC experiment, P074 demonstrated greater toler- e ($P \leqslant 0.05$) than P149 only between the 12- and 24-h flooding treat- nts.

3oth flooding duration and genotype influenced ($P \leqslant 0.005$) the amount cell solutes leached from root segments. Plant-to-plant differences were nd ($P \leqslant 0.005$) within each time and genotype, and there was low variabil- among the 3 root segments within plants.

method. —Xylem water potential provided good differentiation ($P \leqslant 0.005$) ong the 3 genotypes (Fig. 3). As shown by the TTC and EC methods, 4 was more tolerant than P524 or P149. Neither plant age over a period

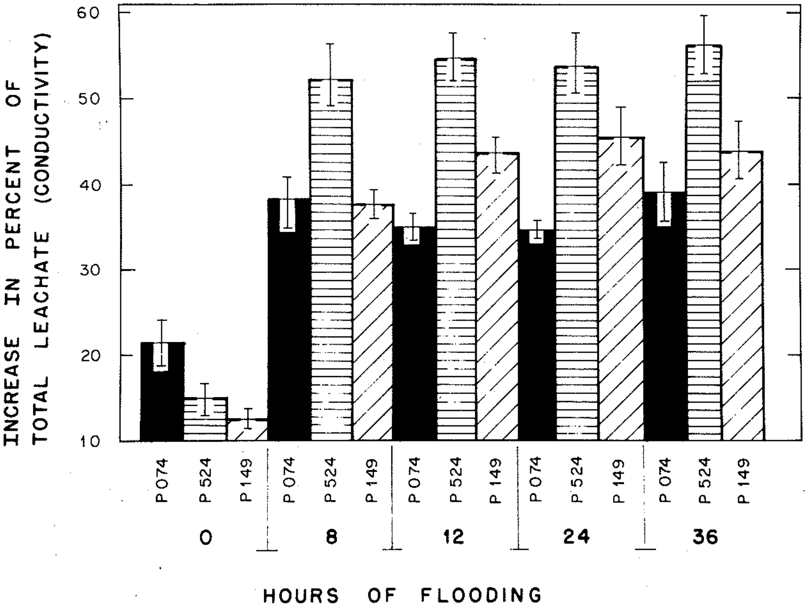

Fig. 2. The effect of flooding upon the percent of total cell solutes leached from se
root segments from bean genotypes P074, P524 and P149. Increasing amounts o
contents leached indicates decreasing flooding tolerance. Each bar represents the
± 1 standard deviation of 9 segments from 3 seedlings.

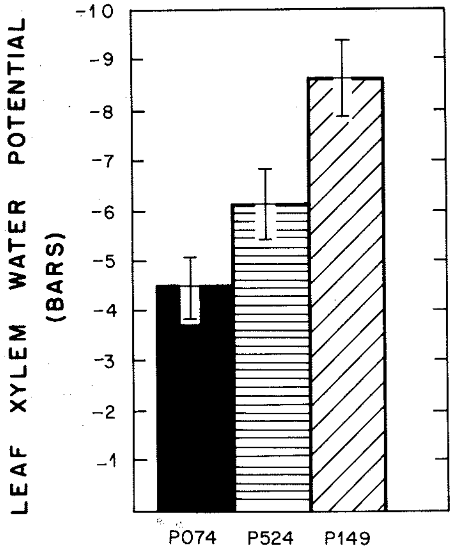

Fig. 3. The effect of 70 h of flooding on the plant xylem water potential of 3 bean
types. Decreasing water potentials indicate decreasing flooding tolerance. Bars rep
mean ± 1 standard deviation of 4 replications of 2 plants each with 3 samples per

2 weeks nor leaf samples within plants caused xylem water potential hange. These observations indicate that (1) a uniform performance within otype may be expected when measuring the xylem water potential of ts with the PC under growth-chamber conditions for a period of about eeks, and (2) the sampling of a single leaf may be expected to provide a esentative indication of the water status of other parts of the leaf canopy. he degree of relationship between the visual scoring and the xylem er potential across the 3 genotypes after 70 h of flooding was as high as 85. This is evidence for the utilization of either method in the deter- ation of tolerance.

iparison among methods. — While P074 and P524 were found to be very rant and sensitive, respectively, by all of the 4 methods, the response of 9 was not as consistent (see Table I). P149 was relatively tolerant accord- to the TTC and EC methods, but sensitive according to the PC and visual ing methods. This apparent inconsistency may be due to the possibility each method measures a particular physiological component contribut- to whole plant tolerance, which conceivably could differ in its expression cause, even between cultivars of the same species (Crawford, 1966). mately, the visual scoring method might be used to compare the effec- ness of the other methods. The precision and sensitivity of the TTC, EC PC methods make them practical indicators of the underlying physiolog- condition of the stressed plant.

or rapid use as screening tools enabling selection of large numbers of otypes in a breeding program, TTC and EC, as now used, may be too rious. They would also require more highly trained personnel. The surement of plant desiccation via the PC might be relatively useful, ever. The mobility of the instrument, and the non-destructivity and atability of the method for each plant make it attractive to the plant der. Although no prior studies regarding its utilization for measuring rlogging damage are known, strong relationships have been found veen water potential measurements and other methods used to evaluate ding tolerance, such as transpiration rates (Regehr et al., 1975), the entage of wilted plants (Rowe and Catlin, 1971), and levels of abscisic and ethylene (Wright, 1977). However, our results indicate that visual ing may also be a satisfactory method. Both the visual and PC methods non-destructive, but they do require larger, well-developed plants and l environmental control during plant growth and flooding. The TTC EC methods, on the other hand, are suitable for use with seedlings, significantly reducing the time and space required to grow plants for ng purposes. Nevertheless, it should be remembered that sensitivity to ding could be different at different plant-growth stages.

ritance study. — In the inheritance study, differences between P524 P149 and among the populations generated from them were non-significant; hence our analysis of them did not proceed further. There considerable phenotypic variability within both P074 and P149, based the visual rating. Only 62% of the P074 plants were rated tolerant, whe 85% of the P149 plants were rated sensitive. Segregation within the F_1 and back-cross populations appeared to fit a normal distribution. Beca sample sizes were small and the genetic constitution of the parents not known, firm conclusions could not be made regarding the inheritance tolerance (Fig. 4). However, back-cross differences ($P \leqslant 0.05$) were fo between (P074 X P149) X P074 and P074 X (P074 X P149). The back-c of the F_1 to P074 appeared to transfer a degree of tolerance which excee that of the F_1. The back-cross of the F_1 to P149 appeared to transf degree of flooding sensitivity greater than that of the F_1.

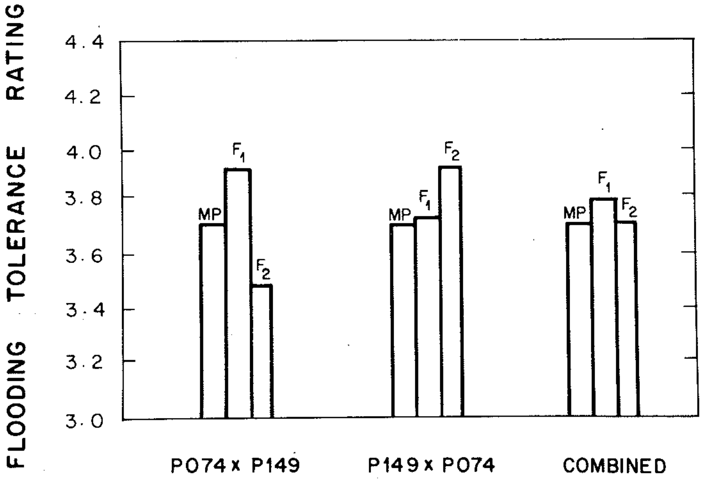

Fig. 4. Mid-parent (MP), F_1 and F_2 mean visual index ratings for flooding toleran reciprocal bean crosses. Increasing index value indicates decreasing flooding tolera as based on a 1—6 scale.

CONCLUSION

Our study shows that several methods can be used to evaluate the de of stress imposed on *Phaseolus vulgaris* genotypes differentially sensitiv soil waterlogging. PC and visual scoring methods were equally effective. T and EC were also useful, but were more time-consuming and gave m variable results. However, the latter 2 methods require only 7-day seedlings, thus permitting the use of larger samples and more efficient of space.

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