UDC 575:630
DOI: 10.2298/GENSR1303679Z
Original scientific paper

APPLICATION OF STRESS SUSCEPTIBILITY INDEX FOR DROUGHT TOLERANCE SCREENING OF TOMATO POPULATIONS

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Zdravković J., Z. Jovanović, M. Djordjević, Z. Girek, M. Zdravković and R. Stikić (2013): *Application of stress susceptibility index for drought tolerance screening of tomato populations.* Genetika, Vol 45, No. 3, 679-689.

Investigation comprised 41 tomato genotypes originating from the population of domestic and domesticated genotypes collected in Serbia and belonging to the tomato collection of the Institute of Vegetable Crop Science, Smederevska Palanka. The aim of collection screening was to choose the genotypes tolerant to drought during plant intensive growth stage, whereby the process of selection would set out to obtain the recombinant genotypes for this abiotic factor. The screening criteria were established for genotype divergence in plant height and shoot-root ratio under conditions of optimal irrigation regime and drought. Divergence was estimated using cluster analysis with Euclidean distance as a measure of distance, with a complete gene attachment to grouping. Drought tolerance is expressed by the stress susceptibility index (SSI). Various results were obtained based on the screening of genotypes grown under optimal and dry conditions. As a measure of stress susceptibility, based on SSI, genotypes having different drought tolerance level were determined. On the grounds of the analyses carried out, 10 genotypes were segregated (G102, G104, G107, G109, G110, G119, G125, G126, G128 and G141) to represent a basis to obtain the recombinant genotypes and to initiate the selection for drought resistance.

Key words: genetic divergence, stress susceptibility index, vegetative stage of growth, tomato

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INTRODUCTION

Tomato (*Lycopersicon esculentum* Mill) is one of the most widely grown vegetables in the world and, therefore, any factor influencing tomato yield has been attracted considerable interest. Among environmental factors, drought is one of the major limiting factors of tomato fruit growth and productivity (BENTON JONES, 1999). Furthermore, current climate change scenarios in Europe predict that drought events during summer and heavy rainfalls during the other seasons will become more frequent during the next decades (GAO and GEORGI, 2008). Thus, a detailed understanding of the determinants of plant stress tolerance and subsequent breeding for increased stress tolerance will play a major role in future agriculture (LOBELL *et al*., 2008). Adaptation measures to mitigate the reduction of yield induced by drought include the production and use of drought-resistant genotypes. The prerequisite to produce resistant genotypes of tomato, as other vegetables, is a better understanding of the plant response and adaptation to drought stress, the improvement of phenotyping, the selection of key-genes involved in the resistance to drought and the evaluation of the impact of resistance on crop yield and quality. These are very difficult tasks because drought tolerance is quantitative traits, with complex phenotype and genetic control. The reaction of plants, including tomato, to drought is the complex phenomenon where the plant response depends on genotypes, the type, duration or intensity of drought and on phenological stage in which drought stress is experienced (CHAVES *et al*., 2003).

The complexity of drought effects on plants and drought tolerance mechanisms explains the slow progress in breeding tomato for drought conditions. The most commercial tomato cultivars are drought sensitive at all stages of development, including seed germination, seedling emergence, vegetative growth and reproduction. Furthermore, tomato stress tolerance is a developmentally regulated state-specific phenomenon, such that tolerance at one stage of plant development is independent of tolerance at other stages. Conventional breeding based on phenotypic selection is further complicated by the fact that several types of abiotic stresses (as high temperature or high irradiance, water and nutrient deficiency) in the field conditions can influence plant’s reactions simultaneously and may have adverse impact on the accuracy and repeatability of investigated phenotypic traits.

The different strategies have been suggested for selecting resistant and relatively resistant genotypes to drought stress. According to ZAMIR (2001) development of exotic genetic libraries consisting of marker-defined genomic regions taken from wild species and introgressed to the background of elite crop varieties will provide a resource for the discovery and characterization of genes that underlie traits of agricultural value. The potential sources of drought tolerance in tomato are found in wild species, including *L. chilense, L. peruvianum, L. pennellii, L. pimpinellifolium, L. hirsutum, L. cheesmanii, L. chmielewskii* and *L. parviflorum* (FOOLAD, 2005). Using this approach GUR and ZAMIR (2004) could demonstrate that introgressed tomato lines carrying three independent yield-promoting genomic regions produced significantly higher yield than then control lines grown under drought conditions.

Breeding for resistance to drought of tomato is also complicated by the lack of fast and reproducible screening techniques. Although a large number of different traits during vegetative and reproductive growth phases have been employed to characterize the physiological and genetic basis of drought tolerance in tomato, it is still difficult to identify drought tolerant genotypes (FOOLAD, 2005). To differentiate the degree of drought resistance between different genotypes, several selection indices have been suggested. One of them is the stress susceptibility index (SSI) which is a ratio of genotypic performance under stress and non-stress conditions (FISCHER and MAURER, 1978). Efforts to overcome the problem of drought and to save water for irrigation of tomatoes were, in the previous period, solved by applying the agro-technical interventions (measures). Partial root drying (PRD) technique has been the subject of research of the members of the research team in the previous period (STIKIĆ *et al.* 2003, SAVIĆ *et al.* 2004, 2006). However, there was a need to include this problem in selection programs of tomato, in order to solve the global problem multidisciplinary: by affecting the physiological processes of growth and creating tolerance in selected genotypes for decreased irrigation conditions (ZDRAVKOVIĆ *et al* 2010).

The aim of presented study was to use the stress tolerance index to screen the drought tolerance of 40 tomato genotypes from "domestic" tomato population collected in Serbia. Analysis was done on the base of two drought-associated traits (plant height and shoot/root ratio) during vegetative phase of plant development. The identified genotypes with increased drought tolerance will be use as starting material in the tomato drought tolerance breeding program.

MATERIALS AND METHODS

Investigation was done with the population of 40 tomatoes (*Lycopersicon esculentum* Mill.) genotypes which belong to the so-called "domestic" populations collected in various parts of Serbia. The investigated population is a part of tomato germplasm stored and maintained at the Institute for Vegetable Crops in Smederevska Palanka. The experiments were done in controlled glasshouse conditions in the Institute. The experimental design was a randomized complete block system with 4 replicates and 10 plants per replicate. Plants were raised from seed and transplanted into 800 pots (one plant per pot) filled with 600cm^3 of commercial compost (Biolan C1-B, Finska) in a controlled glasshouse condition. After transplantation, all plants were irrigated daily to full pot holding capacity, volumetric soil water content of 35%. The soil water content was measured daily using TDR probes (time domain reflect meter, TRASE, Soil Moisture Equipment Corp., USA).

Ten days after transplantation, plants were subjected to following treatments: 1. full irrigation in which the whole root system was irrigated daily at 9:00 h to reach a value of maximal field capacity (35%) and 2. drought treatment where the soil water content was reduced to the soil water content of 20.9%. After 10 days of drought treatment and in the phase of intensive vegetative growth, measurement of plant height was done and samples of shoots and roots were collected for measuring dry matter. Dry matter content was determined after drying the shoot (leaf and stems) and root samples to a constant weight in a drying oven at 80^{o}C. On the base of these results ratio between shoot and root DW was calculated.

To evaluate the drought tolerance of investigated genotypes the stress susceptibility index (SSI) was determined. According to the FISCHER and MAURER (1978) method the SSI was calculated as differences in the results obtained for stress (drought) and non-stress (control) conditions by using the following equations:

$SSI = [1 - Yp/Ys]/SI$

$SI = [\ 1 - /MYs/MYp]$ – stress intensity

In the above equations, Yp is the mean values for the investigated trait under non-stress conditions, Ys is mean trait value under stress conditions, MYp is mean trait value of all investigated genotypes under non-stress conditions and MYs is mean trait value of all genotypes under stress conditions.

Multivariate cluster analysis was also employed as a selection criterion in order to express the divergence of the investigated tomato genotypes according to phenotypic characteristics. Divergence was estimated using Euclidean distance with complete genes' attachment to grouping (STATISTICA 8.0; StatSoft, INC. (2007), data analysis software system, www.statsoft.com).

RESULTS AND DISCUSSION

The investigated 40 tomato genotypes were collected from Serbia over a longer period of time. Among them, it was a large variation in investigated vegetative traits (plant height and shoot/root ratio) in optimal and also drought conditions (Table 1). Drought induced a significant reduction in plant height of treated plants as compared to control (reduction varied from 12.32% in G110 to 40.66% in G115).There were also significant differences among genotypes in respect to shoot /root dry weight ratio, which also demonstrated high diversity among them that enabled screening for drought-tolerant genotypes. These results showed the differential response of root growth to drought in investigated genotypes. In some genotypes (G110, G124, G126) under a condition of drought stress shoot to root ratio decreased compared to fully watered plants. Decreased values of the shoot/root ratio indicated that in some genotype's drought caused a promotion of root growth.

Table 1. The effects of drought (D) and full irrigation (FI) on shoot/root ratio and plant height in investigated tomato genotypes
a) Determinant genotypes

Genotypes	Shoot/root ratio		Plant height (cm)		Plant height reduction (%)
	FI	D	FI	D	
G101	2,07	3,06	108	93,8	13,15
G102	1,88	6,69	76,8	59,6	22,40
G103	2,81	4,52	104	74,2	28,65
G104	2,36	3,15	82,2	72,2	12,17
G105	2,46	5,04	84,6	68,8	18,68
G106	5,03	12,14	75,4	53,2	29,44
G107	11,21	15,18	77,4	53	31,52
G108	13,04	9,07	23,2	19,4	16,38
G109	7,21	13,59	69	58,8	14,78
G110	4,07	3,11	69,8	61,2	12,32
G111	5,03	9,29	72	48,8	32,22
G112	5,63	8,49	56,8	40,6	28,52
G113	5,22	10,65	55,2	39,2	28,99
G114	3,46	6,66	86	62,4	27,44
G115	4,62	11,78	78,2	46,4	40,66
G116	6,38	11,78	71,75	47,6	33,66
G117	6,27	11,79	69,8	49,4	29,23
G118	3,59	7,39	81,8	55,6	32,03
G119	5,16	6,64	74,32	51,2	31,11

b)Indeterminate genotypes

Genotypes	Shoot/root ratio		Plant height (cm)		Plant height reduction (%)
	FI	D	FI	D	
G120	5,46	12,43	97,2	66,8	31,28
G121	7,08	10,59	89,6	56,6	36,83
G122	6,90	12,06	77,4	50,76	34,42
G123	4,36	9,82	78,8	58,8	25,38
G124	6,76	5,89	93,8	65	30,70
G125	6,12	6,50	80,6	59,2	26,55
G126	4,55	4,21	118,6	94	20,74
G127	6,89	12,18	101,4	83	18,15
G128	6,50	6,45	106,6	75	29,64
G129	4,18	9,02	111	87,8	20,90
G130	2,13	7,09	141,8	105,8	25,39
G131	1,91	5,18	124,4	87,8	29,42
G132	2,22	4,41	137,8	100,6	27,00
G133	1,85	5,39	141,2	105	25,64
G134	2,07	6,93	116	81	30,17
G135	2,62	4,51	121	95,6	20,99
G136	3,30	7,78	130,8	80	38,84
G137	3,56	7,27	111,8	80	28,44
G138	2,76	6,21	121	89,4	26,12
G139	2,29	3,38	106,2	89,6	15,63
G140	6,09	15,52	126,4	78,25	38,09
G141	5,78	6,57	128,32	106,6	16,93
Stress intensity (SI)	-0,708		0,265		

The evaluation of reaction to drought of investigated genotypes was also done by using of SSI index. There are a number of ways to determine stress intensity, but this one has been established as the most useful in the comparative analysis among the four indicators of stress effect (ILKER *et al*., 2011). Through this index, more or less tolerant genotypes to drought can be specified. When the amount of SSI is less, the tolerance of genotype to drought stress will be higher. Graph 1 show the SSI values for plant height which was in the range from 0 to 1.6. The SSI values between 0.4 and 0.6 of several genotypes (G102, G104, G109, G110, and G139) indicated that these genotypes can be considered highly tolerant to drought caused stress. Higher SSI values (0.6 to 0.8 ranges) which were still within the limits can be considered as values for moderate drought tolerance. These values were calculated for following genotypes: G105, G126, G127, G129, G135 and G141.

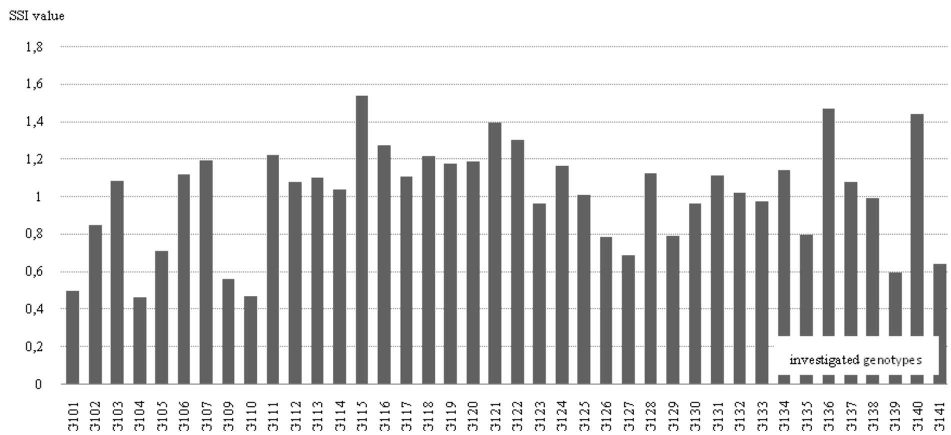

Fig 1. The stress susceptibility index calculated on the base of plant height values.

The SSI values for the shoot/root ratio where in the range from 1 to 4 (Graph 2). The values between the -0.5 and 0.5 were considered as values indicating the genotypes with the tolerance to drought (G105, G107, G110, G119, G124, G125, G126, G128, and G141). The SSI values for four genotypes (G110, G124 and G126) were negative, which indicated that under drought conditions, the development of the root system in these genotypes was more intensive than shoot growth.

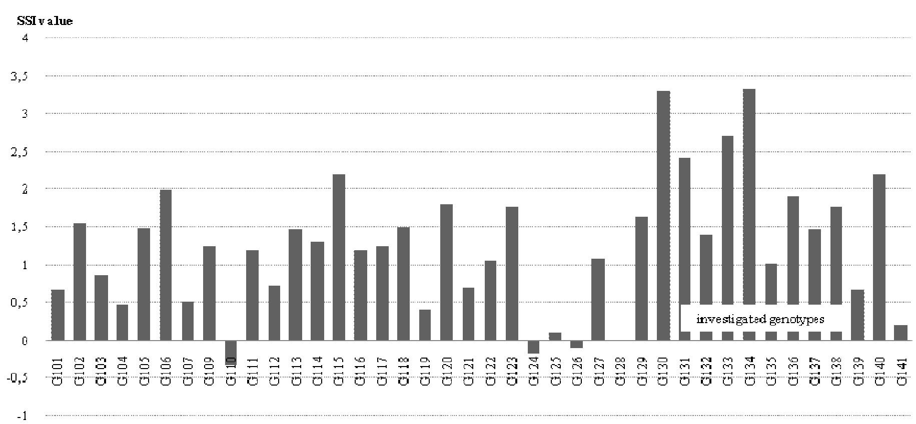

Fig. 2: The stress susceptibility index calculated on the base of shoot/root ratio values.

Plant growth rate is generally inhibited by soil drying, but many results confirmed that root growth is less influenced by the soil water deficit than that of the shoot. The maintenance of, or even promotion of, root growth during soil drying can provide several advantages, such as a better exploitation of soil nutrients and water when environmental conditions are less favorable (SHARP *et al*., 2004). The increase in root dry mass in our experiments was most likely attributable to stimulated secondary root initiation and root growth. The similar trend of shoot and root growth and dry matter accumulation has been recorded by other authors in tomato plants grown under drought or deficit irrigation conditions (MINGO *et al*., 2004: PROKIC and STIKIC, 2012). There is also some consistency in results concerning the root's characteristics. Drought resistance of tomato related wild species *L. chilense* was attributed to its deep vigorous root system. In contrast the results for the "drought-tolerant *L. pennellii* accession LA716 showed a limited and shallow root system. In the basis of drought tolerance of this genotype is the specific succulent leaf structure with allows reduction of transpiration during the drought period (FOOLAD, 2005).

The final estimation of genotypes to be included in the selection program for drought tolerance involved the use of cluster method. The aim of the analyses was to classify different genotypic groups in similar classes. The dendrograms of plant height mean values for genotypes grown under the optimal water regime showed the distribution of genotypes into two groups (Fig 3). The formation of three (I, IIa and IIb) groups indicates the existence of divergence of population selected for the tomato breeding program for drought tolerance. The largest number of genotypes under the irrigation regime belonged to cluster I (17 genotypes), then to cluster IIa (14), and to cluster IIb (9).

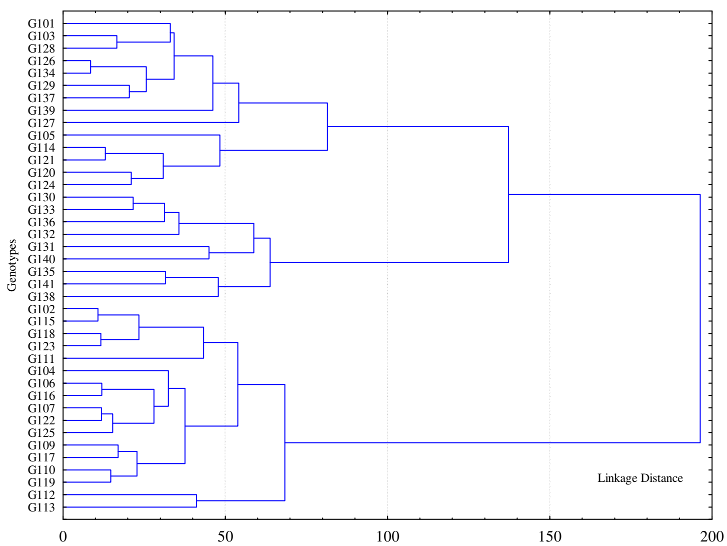

Fig 3. Dendogram of genetic distance of genotypes (Euclid distance) based on plant height of genotypes under full irrigation conditions.

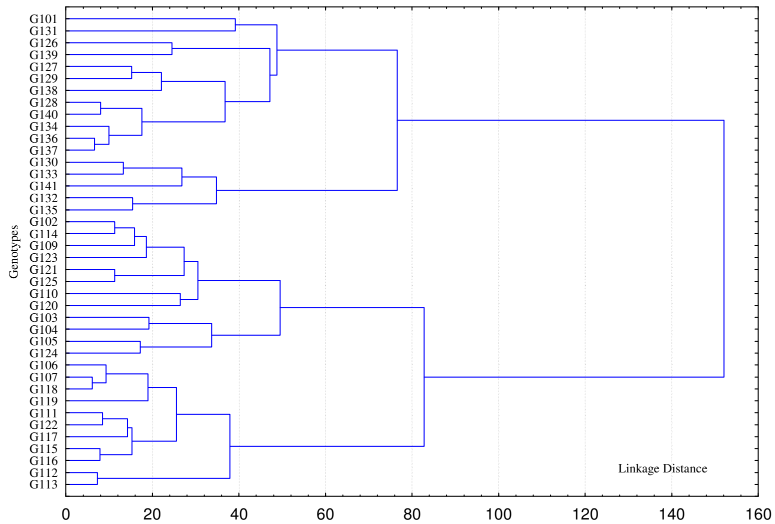

Fig 4. Dendogram of genetic distance of genotypes (Euclid distance) based on plant height of genotypes under drought conditions.

The dendrograms resulted from cluster analysis of genotypes under drought conditions stress conditions was not diametrically different from the dendogram of plants under the optimal water regime. All genotypes were also located into two larger groups (I and II), and each cluster contains less two smaller groups (Ia,Ib and IIa, IIb, respectively), Fig 4. However some genotypes were distributed into different clusters (G124, G120, G121,G114, G105 and G103) which only indicate that they responded alike by plant height to drought impact. The largest number of genotypes is found in clusters Ib and IIb (12 genotypes), Ia (11 genotypes), and smallest group IIa (5 genotypes).

Of special importance in drought tolerance breeding program is phenotypic characterization of useful germplasm. In the literature there is a limiting data concerning this issue. More data are available for tomato resistance to another stress factors as salt or temperature (FOOLAD, 1999). The majority of available selection criteria for screening or breeding tomatoes for drought resistance come from the research done with tomato wild relatives. In these experiments different tolerance indices were used: including dry weight (DW) of shoot and root, root length, root morphology, leaf rolling, flower and fruit set, fruit weight, fruit yield, WUE, recovery after re-watering, stomatal resistance, plant survival, leaf water potential, leaf osmotic potential, osmoregulation, transpiration rate, photosynthetic rate, enzymatic activities etc. (FOOLAD, 2005). However, these traits should be further checked in tomato inbred lines or cultivars under drought conditions. There are also results done by genotypes which not belong to the exotic germplasm. These results indicated that in these genotypes, genes responsible for drought resistance may be found (MANOJ and UDAY, 2006, 2007). Recently, WAHB-ALLAH *et al.* (2011) have screened drought resistance of four tomato cultivars based on several vegetative and fruit traits (plant height, stem diameter, leaf and fruit dry matter, fruit setting and yield) under drought conditions. Significant differences among genotypes were found for almost all of these traits (except stem diameter), suggesting that they could be taken into account when selecting for tomato drought tolerance.

Phenotypic analysis of genotypes for plant height has shown a high level of heterogeneity and thus, may represent a desirable source of genetic variability. Dendograms constructed for optimal irrigation regime and for drought conditions revealed two population groups. These groups are already known as the groups of determinate and indeterminate types of shoot growth habit. Their heterogeneity within a sub-cluster indicated diversity and possibility to choose suitable genotypes.

CONCLUSIONS

The results of stress susceptibility index and grouping of genotypes on the base of plant height and shoot/root ratio, lead to the conclusion that 11 genotypes (G102, G104, G107, G109, G110, G119, G125, G128 and G141) exhibited better drought tolerance characteristics than others from “domestic“ population. Thus, they and could be suitable initial genetic material in the selection program for tomato drought tolerance. However, broader conclusions can be obtained only after molecular analyses of these genotypes.

ACKNOWLEDGEMENT

This study was supported by Serbian Ministry of Education and Science (project TR 31005 and TR 31059).

Received July 22th, 2013
Accepted October 05th, 2013

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PRIMENA INDEKSA OSETLJIVOSTI NA SUŠU ZA SKRINING OTPORNOSTI NA SUŠU POPULACIJE PARADAJZA

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Izvod

Ispitivanje je izvršeno na 41genotipu paradajza poreklom iz populacije domaćih i odomaćenih genotipova prikupljenih iz Srbije, a pripadaju kolekciji paradajza Instituta za povrtarstvo u Smederevskoj Palanci. Skrining kolekcije imao je za cilj da se izvrši izbor genotipova tolerantnih na sušu u vegetativnoj fazi intenzivnog porasta biljaka, čime bi se započeo program selekcije na dobijanje rekombinovanih genotipova prema ovom abiotskom faktoru. Kriterijumi za skrining bili su divergentnost genotipova za visinu biljke i odnos izdanak/ koren u uslovima: optimalnog režima navodnjavanja i suše. Za ocenu divergentnosti korišćena je klaster analiza sa Euklidovom distancom kao merom udaljenosti, sa kompletnom vezanošću gena za formiranje grupa. Tolerancija na sušu izražena je indeksom osetljivosti na stres (SSI-stress susceptibility index). Različiti rezultati su dobijeni na osnovu skrininga genotipova koji su gajeni u optimalnim uslovima i u suši. Kao mera osetljivosti na stres prema indeksu osetljivosti definisani su genotipovi sa različitim nivoom tolerancije na sušu. Na osnovu izvedenih analiza izdvojeno je 10 genotipova (G102, G104, G107, G109, G110, G119, G125, G126, G128 i G141) koji će predstavljati bazu za dobijanje rekombinovanih genotipova i početak selekcije na otpornost na sušu.

Primljeno 22. VII 2013.
Odobreno 05. X. 2013.