



Original Contribution

SCREENING CAPSICUM GENOTYPES FOR INCREASED DROUGHT TOLERANCE BY IN VITRO POLLEN GERMINATION AND POLLEN TUBE LENGTH

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ABSTRACT

Yield realizing in vegetable crops depends on several reproductive processes that include pollen fertility as well as pollen tubes germination and growth. The study was conducted as the pollen characteristics fertility and viability were used, as well as the productivity for evaluation of drought tolerance in four pepper genotypes *Capsicum annuum* L. From the conducted cytological analyses was found that decreasing the water standard with 50% exert negative influence over the male gametophyte and the pollen fertility decreases with up to 37.4% average. More sensitive to drought showed to be the plants from both cultivars Pazardzhishka kapia and Albena, in which lower productivity and lower restorable potential were found in comparison with those found in the mutant lines No.1936 and No.1917. Increased tolerance showed the male gametophyte of line No.1936. After a drought in it were found the highest values for pollen viability – 33.4% average pollen germination and 438.7 μ m pollen tubes length which was with proven mathematical difference compared with the rest of the genotypes. These results showed that pollen fertility analyses and pollen viability analyses in drought plants could be used for evaluation of the drought tolerance.

Key words: Drought tolerance, pollen fertility, fruit yield, pepper *Capsicum annuum* L.

INTRODUCTION

On a world scale, a water stress is accepted as an explicitly serious problem that inhibits plants productivity (1, 2). Bell pepper (*Capsicum annuum* L.) is one of the most important cultures in the world (3) and is one of the most pliable to water (4-6). The drought in the pepper production, impose great yield decreasing and quality of the sowing with significant economic losses up to 70% (6, 7).

Various physiological criteria have been proposed by several authors in different crops however, there is limited information on the use of drought tolerance indices on important agronomic traits of pepper (8-12). Plant reproductive development is more sensitive to environmental stresses such as drought (13-16) compared to vegetative growth and male

Gametophyte development it is the most sensitive stage in the plant life cycle being easily affected by stressful environment (17, 18).

The aim of this study was to evaluate the drought resistance through analysis of the male gametophyte and the productivity in four pepper genotypes.

MATERIALS AND METHODS

The experiments were conducted in glasshouse Venlo type in the MVCRI, Plovdiv, Bulgaria with four pepper (*Capsicum annuum* L.) genotypes – two cultivars Pazardzhishka kapia and Albena and two mutants line No.1917; No.1936. The plants will be cultivated as a substrate culture without heating. The trial will be put by the block method in three repetitions as in each repetition there will be by 4 plants.

During the bud formation-blossoming period, the plants were exposed in two watering regimes: the first regime – Control 100% watering norm (the number of the watering

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will correspond to the perceived for that production technology) and the second regime – drought 50% watering norm (the number of the watering will be reduced with 50% towards the perceived for that production technology). Pollen fertility was studied in five or more flowers from each plant in the different genotypes. Temporary preparations are made and they are colored with a mixture of 4% acetocarmine and glycerine in correlation 1:1. At least 100 pollen grains are enumerated from different visual fields. The percentage of the colored (fertile) and non-colored (sterile) pollen is defined.

The male gametophyte viability was characterized by two indices – pollen grain germination (x, %) and pollen tube elongation (l, μm). The method of the hanging drop was used for the pollen grain germination. The pollen was sown in Petri dishes with nutritive medium containing agar, saccharose, H_3BO_3 and CaCl_2 . The percentage of germination and the pollen tube lengths were determined 24h after placing the Petri dishes in thermostat at 26°C . Two or three drops from each flower were used for the pollen germination. Minimum of 100 pollen grains per drop was counted.

The quantitative drought tolerance criteria were calculated according to (19): Tolerance index (TI) = $Y_p - Y_s$; Mean Productivity (MP) = $(Y_p + Y_s)/2$ and Percent Injury (I %) = $(Y_p - Y_s)/Y_p \times 100$. Y_p is mean trait performance under control and Y_s is mean trait performance under watering regime.

RESULTS AND DISCUSSION

The investigation of the influence of drought over the male gametophyte of the four pepper genotypes showed the negative effect of that abiotic stress over the pollen fertility and viability in all of the studied plants.

Pollen fertility in dry up genotypes decreases average with from 20.3% in the mutant lines No.1936 and 1917, to 37.4% in Albena variety in comparison with the control plants. The increased resistance to water deficit in the male gametophyte of the mutant lines showed during the entire studied period (**Figure 1**) as lowest fertility values were recorded during the formation and ripening of the first fruits – in September. That corresponded to the work of (20, 21), who claim that a critical period for the male gametophyte is the early fruiting.

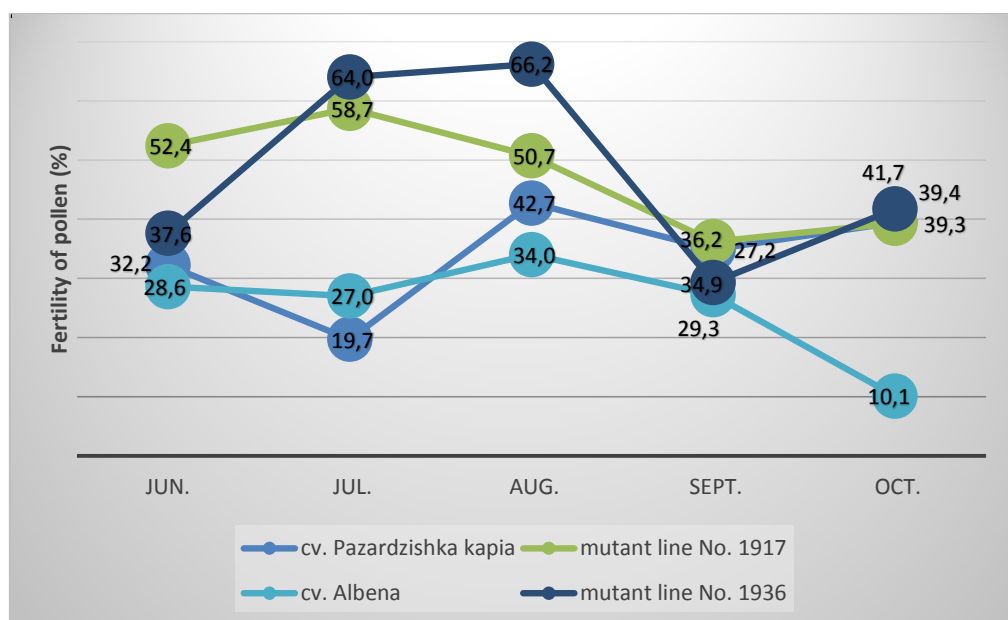


Figure 1. Fertility of pollen (average %) of genotypes under 50% water regimes.

The pollen kept its fertility in the normal watering in over than 95.2% from the reproductive organs of the studied varieties and lines (**Figure 2**). The water deficit had lethal

effect in from 37.7% to 59.4% of the investigated buds and flowers in line 1917 and Albena variety as after treating the reproductive organs faded and fall down.

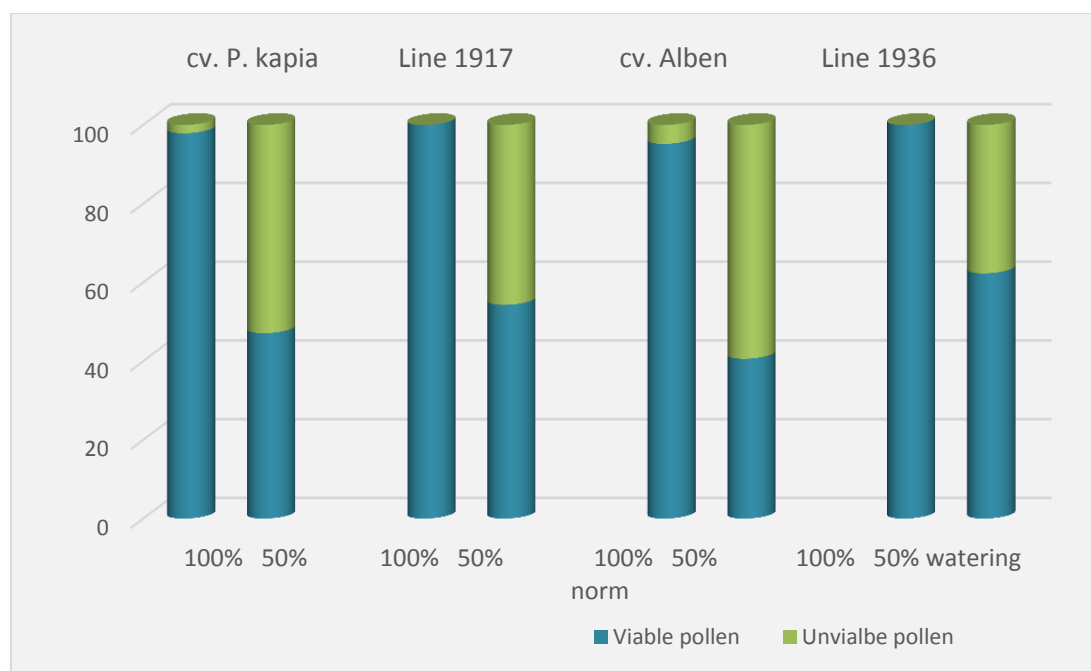


Figure 2. Buds and flowers (%) with viable and unviable pollen.

Drought had a strong negative effect over the in vitro germination of the pollen grains over the nutrition environment and pollen tube growth. The percentage of the germinated pollen was from 16.6 average in Albena

variety to 33.4 in line 1936 (**Figure 3**). Albena and Pazardzhishka kapia varieties showed the highest pollen sensitivity with lowest restoring potential by that index from the studied genotypes 49.1% and 51.4 respectively.

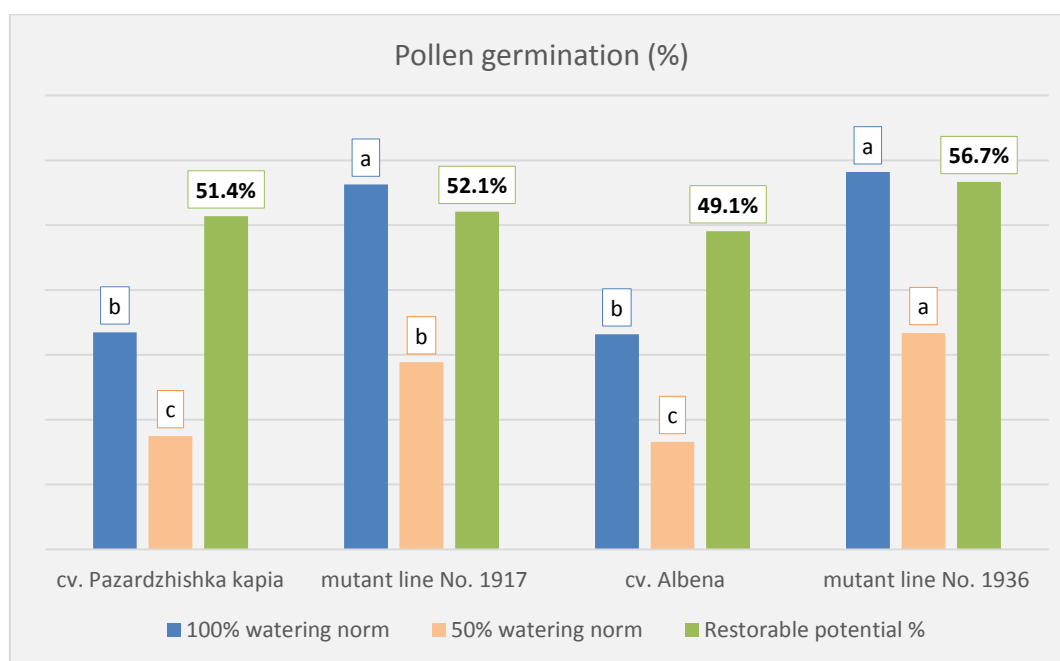


Figure 3. Pollen germination and restorable potential of genotypes under 100% and 50% water regimes.

Certain tolerance of the male gametophyte was found in the mutant lines 1917 and 1936, which showed highest indices values defining the pollen viability with mathematically proven difference toward the rest of the genotypes (**Figures 3, 4**). The pollen germination was 33.4% average for line 1936

and 28.9% in line 1917 and the pollen tubes length – 438.7 μ m and 262.8 μ m average. The mutant line 1936 stands out with the highest restoring potential – 56.7% for the pollen germination and 79.5% in respect of the elongation of the pollen tube.

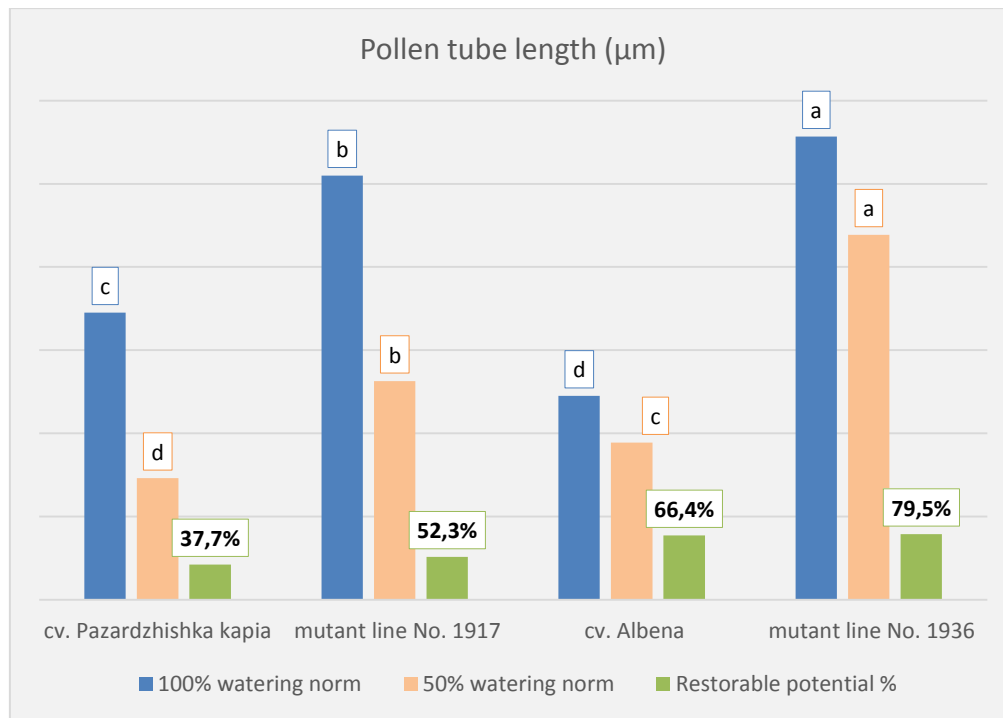


Figure 4. Pollen tubes length and restorable potential of genotypes under 100% and 50% water regimes.

The water stress response was obvious when reducing the watering norm with 50% (**Tables 1, 2**). Decrease of the total fruit production was with 30.3% average and decrease of the first quality production with 30.2% average. That corresponds to the results received by (22) and (23) in which the shortage of water decreases the fruit mass with 34.7% in comparison with the control watering. The water deficit exert

the strongest effect over the productivity of Pazardzhishka kapia variety where the total yield decreased with 46.0% average and the first quality production with 39.7% as the quantity of the fruits with small size and sun burn and rotting – 287.2g/plant average (**Tables 1, 2**). The constant water deficits over pepper significantly increase the fruits that cannot be sold with up to 67% (24).

Table 1. Fruit yield - productivity (g/plant) and drought tolerance criteria

Genotypes	Fruit yield / Productivity					
	Yp control	Ys stress	Potential (P)	Tolerance index (TI)	Mean productivity (MP)	Percent injury (I%)
cv. Pazardzhishka kapia	643,7	339,7	52,8	304,0	491,7	47,2
	661,6	379,9	57,4	281,7	520,8	42,6
	663,9	344,0	51,8	319,9	504,0	48,2
Mutant line No. 1917	694,4	516,0	74,3	178,5	605,2	25,7
	656,2	498,2	75,9	158,0	577,2	24,1
	665,6	501,6	75,4	164,1	583,6	24,6
cv. Albena	578,9	435,3	75,2	143,6	507,1	24,8
	528,5	415,4	78,6	113,2	471,9	21,4
	515,3	455,0	88,3	60,2	485,1	11,7
Mutant line No. 1936	713,2	507,8	71,2	205,4	610,5	28,8
	744,4	521,7	70,1	222,8	633,1	29,9
	708,0	503,4	71,1	204,7	605,7	28,9

Table 2. Fruit yield - First quality (g/plant) and drought tolerance criteria

Genotypes	First quality fruit yield					
	Yp control	Ys stress	Potential (P)	Tolerance index (TI)	Mean productivity (MP)	Percent Injury (I%)
cv. Pazardzhishka kapia	372,4	235,3	63,2	137,2	303,8	36,8
	366,8	209,9	57,2	156,9	288,3	42,8
	368,5	223,0	60,5	145,5	295,8	39,5
Mutant line No. 1917	404,8	300,8	74,3	104,1	352,8	25,7
	439,8	282,8	64,3	157,0	361,3	35,7
	407,0	295,8	72,7	111,3	351,4	27,3
cv. Albena	322,6	215,2	66,7	107,4	268,9	33,3
	303,1	223,2	73,6	79,9	263,1	26,4
	326,7	256,6	78,5	70,1	291,6	21,5
Mutant line No. 1936	413,6	300,7	72,7	112,9	357,2	27,3
	425,8	326,0	76,6	99,8	375,9	23,4
	405,5	310,6	76,6	95,0	358,0	23,4

For studying the reaction of drought over the pepper plants and selection of genotype with increased tolerance were conducted a number of studies with results in different directions. From a glasshouse study on 'Sonar' sweet pepper (*Capsicum annum* L.), (25) reported increased fruit dry mass, while (26) observed a 30% reduction in both fruit fresh and dry masses in the field-grown 'Quadrato d'Asti' sweet pepper. Like (27-29) we claim that a selection for drought tolerance only by yield is an unreliable method and complex evaluation is needed when formulating the breeding criteria for tolerance to drought in pepper.

The lower fertility and viability of the pollen in the current study was accompanied by reducing the productivity of the investigated genotypes which gave us a reason to accept the gamete breeding as an effective means for evaluation of pepper for increasing its drought tolerance. As a confirmation of that are the studies of (30-32). They found a connection between the pollen colouring and yield in tomatoes, green beans, and pepper as well as higher pollen fertility in resistant genotypes in comparison with those sensitive to the abiotic stress.

Drought tolerance criteria of the productivity in pepper in conditions of water stress are shown in **Tables 1 and 2**. The lowest tolerance index was obtained for the total yield followed by a number of fruits from the first quality in Albena variety. The highest percentage of damage was reported in the total yield (46%

average) followed by first quality production (39.7% average) of Pazardzhishka kapia. That confirms that the total productivity was the most affected by the drought and because of that it needs a significant improvement for the drought tolerance. Mean productivity was also higher for the total yield in comparison to the yield from first quality in the studied genotypes with values from 488.1% in Albena variety to 616.4% in the mutant line 1936. We agree with (29) and consider that the three indices for drought tolerance TI, MP and I% were appropriate in identifying the productivity and could be used in screening and breeding criteria formulating for drought tolerance in pepper.

CONCLUSIONS

1. Decreasing the watering norm with 50% simulated the water stress and was effective in investigating the pollen reaction and productivity in pepper plants. That could be used for further improvement of the crop in the field of drought tolerance.
2. Drought influenced negatively over the male gametophyte as it decreases pollen germination and pollen tubes elongation with 21.2% average and 155.0µm for all of the investigated genotypes.
3. Mutant line No.1936 was defined as a line with increased water deficit tolerance in the treated plants of which were found highest pollen viable indices – germination 33.4% average and pollen tubes length 438.7µm average and highest productivity – total yield 510.9g/plant average and first quality

production 312.4g/plant. That positive correlation gave us reason to define the gamete breeding as an effective means for increasing pepper drought tolerance.

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