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Influence of sodium azide on morphological traits of Proso Millet (*Panicum miliaceum* L.) Genotypes

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ABSTRACT

Objective of the present investigation was the study the effectiveness of sodium azide (NaN_3) on the morphological traits of the initial breeding materials of millet (*Panicum miliaceum* L.). The seeds were treated with different concentrations of sodium azide (0.1; 0.2; 0.3 and 0.4%). Number of morphological mutant were observed visually and recorded. According to the obtained results the stimulating effect of mutagen is clearly determined, the higher the level of the concentration of mutagen, the stronger the decrease in seed germination. In general, lower 0.1 mM concentration of sodium azide was most effective. Different concentrations of sodium azide inhibited most agronomic traits such as germination, plant height, panicle length, panicle weigth and one thousand seed weight of proso millet in the field conditions.

Key words: *Proso millet, Mutagenesis, Sodium azide, Treatment, Germination, Effectiveness*

Introduction

Mutagenesis is widely used in plant breeding for the creation of genetic variability of crops that is absent in the gene pool (Ambli *et al.*, 2014). More than 3000 varieties of cultivated plants have been created in the world at the moment using experimental mutagenesis. The method is quite effectiveness in plant breeding all the main economically valuable traits. Achieving the same result using by other methods is much more difficult or not advisable in some situations (Morgun *et al.*, 1995). Various mutagens are used to produce induced mutations in plants. It is known series of mutagenic substances belonging to different classes of chemical compounds. Among them are important mutagens such as ethylene nimine, ethyl methanesulfonate, diethyl sulfonate, 1,4-bis-diazoacetyl butane, nitrosoalkylurea, sodium azide and other substances. Many studies have shown that chemical mutagens are significantly more effectiveness than physical. If under the influence of radiation in plants up to 10-15% of viable changes occur, then using of chemical mutagens allows you to get them up to 30-60%. Widely using of the mutational breeding method in many countries in the world has confirmed its high effectiveness in solving various problems of plant breeding. Mutants are often of great breeding value, as they may have new, previously unknown useful traits. In addition, with the help of mutagenesis, it is possible to overcome the technical difficulties that arise when crossing small-flowered crops, such as proso millet (Kumar *et al.*, 2003; Kumar *et al.*, 1996; Kumar *et al.*, 2010; Maduli *et al.*, 2007; Mehta *et al.*, 1994; Singh N.K. *et al.*, 2009). In connection with the discovery of chemical supermutagens that showed high activity in experiments with different biological objects, including proso millet, breeders' interest is increased in experimental mutagenesis.

Common millet or millet (*Panicum miliaceum* L., 2n = 4× = 36) is an ancient crop of the genus *Panicum* and includes more than 400 species (Roshevits 1980). Proso millet has been cultivated for more than 5000 years ago in Central and East Asia (Ho, 1977). Common millet has food, feed, reserve and strategic importance and is cultivated in 30 countries of the world, including in 18 countries of Europe. At present, the main producers of common millet are: the Russian Federation, India, China, the USA and Ukraine (Zotikov *et al.*, 2012; Sidorenko *et al.,* 2015). Due to its precocity, drought tolerance, productivity and other valuable biological and economic traits, this culture can be widely used in production (Tsygankov *et al.,* 2006). Common millet by drought- and heat tolerance is one of the first places among other spring crops, which is very valuable for arid regions. For the conditions of the dry-steppe zone of the Republic of Kazakhstan, it is one of the most adapted crops (Fedulov *et al.,* 2015). Objective of the present investigation was the study of the effectiveness of sodium azide (NaN_3) on the morphological traits of proso millet genotypes.

Materials and Methods

Plant material

Objects of the research were cultivars and samples of proso millet of local and foreign selection. Dry seeds of foreign genotypes (Saratov 6, K-10342 and K-3742) proso millet were obtained from the *N.I.* Vavilov *Institute of Plant Genetic Resources* (VIR). Domestic varieties such as Yarkoye 6 and Shortandinskoye 7 were also included in the experiment.

Treatment with mutagens

The treatment with chemical mutagen was conducted out in the laboratory conditions according to the method using original seeds (Esson *et al.,* 2018). Before the experiment, proso millet seeds were sterilized with 90% alcohol for two minutes to destroy harmful microflora on the surface of the grains, then the seeds were washed twice and presoaked in distilled water for 4 hours in order to allow rapid and efficient diffusion of mutagens into the seeds. After the seeds were soaked in an aqueous solution of sodium azide of various concentrations (0.1%; 0.2%; 0.3%; 0.4%) at an exposure of 4 hours. Then the solution was drained, the treated seeds were washed thoroughly in running tap water to remove the residual effects of mutagens several times and placed in a thermostat at a temperature of 27 °C. In each variant, 50 pcs of seeds were processed in triplicate for each sample. The control variant was germinated in distilled water. On the seventh day, seed germination, the length of seedlings and roots, number of roots were determined. Mean values (M) and standard deviations (m) were calculated for each samples using Microsoft Excel 6.0.

Field experiment

The experiments were laid in the field conditions of A.I. Barayev research and production centre for grain farming. Treated and untreated seeds with different concentration of sodium azide were thereafter taken to the field and sown on the 15th of May 2019. Each treatment was replicated four times. The experiment was conducted during between June-September 2019. Sowing was carried out according to the methodology of state variety testing of agricultural crops (1985) and the Field Experience Methodology (Dospechov, 1985). 1000 seeds were counted and weighed using electric weighing balance.

Results and Discussion

Determination of the effectiveness of sodium azide on the growth parameters of proso millet seedlings in laboratory conditions

Main step in studying the effect of mutagen on plant growth and development is the establishment of effective doses and the duration of exposure of the treated object. In accordance with published data, the influence of concentration of sodium azide solution in the reagent solution on depends on the pH and the most optimal is pH-3 (Gruszka *et al.*, 2012). Therefore, proso millet seeds were treated with an aqueous solution of sodium azide at pH-3 in various concentrations. *Seed germination*, root and seedlings length *of 7-days old* plants *were* measured to analyze the effectiveness influence *of* mutagen *on plant growth.* As a result laboratory studies significant differences in morphometric indicators, depending on the concentration of sodium azide was revealed. It was shown that seeds treated with mutagen had lower germination compared to control values. In the control samples, the seed germinating viability fluctuated from 43.3 to 100%; while in the experimental treatments, it was decline depending on the mutagen concentration respectively, as of 0.1% – from 23.3 to 43.3%, at 0.2% from 13.3 to 26.6%, at 0.3% from 0 to 13.3% (Table 1).

As shown in the presented data, high concentration (0.4%) of mutagen had inhibitory effectivness on seed germination; all samples had no seedling, except for Yarkoye 6 (13.3%), which indicates the high efficiency of this concentration. After seed treatment the germination of varieties with mutagens have depended on the variety. For example, the germinating viability of seeds in the variety Shortandinskoe 7 is varied from 0 to 26.6 depending on the concentration of mutagen, while the variety Yarkoe 6 had a germination rate of 13.3 to 33.3%. Seedlings length is reduced in all mutagens to compared with the control samples (Table 2).

The length of the shoots in 7-day sprouts of millet was on average between 6.5-12.2 mm in control variant. At the concentration 0.4% sodium azide the varieties Shortandinskoye 7, K-3742 and Saratovskoe 6 showed higher lethality. In comparison with control variant the stress caused by different concentrations (75, 100, and 150 mM) of mutagen also inhibited the growth of roots of the studied experimental samples of millet (Table 3).

At 0.1% concentration of mutagen, the root length varied from 2.9-3.5 in studying samples, while at 0.2% concentration this indicator ranged from 0.6 to 3.2. At the high concentrations 0.3 and 0.4% of mutagen the growth of millet roots were decreased. Significant differences from the control by seed germination, by the length of seedlings and roots were observed in all studied samples. The average laboratory of seed germination sprouts length and roots in the studied samples at all mutagen concentrations turned out to be lower than the control (Figure 1).

At 0.1% concentration of sodium azide the germination was quite high and averaged 31.3%. According to the obtained results the stimulating effect of mutagen is clearly pronounced, the higher the level of the concentration of mutagen, the stronger the decrease in seed germination. In general, lower 0.1 mM concentration of sodium azide was most effective. This may be due to the failure in proportional increase of mutation frequency induced at higher treatments (Bashir *et al.,* 2013). Similar result was reported in chickpea (Wani, 2009), and sunflower (Kumar *et al.,* (2010).

The effect of sodium azide on the agronomic traits of proso millet in the field conditions

The germination percentage was counted on the

Table 1. Efficiency of sodium azide on proso millet germination (%)

Genotypes	Treatments, %									
	0		0.1		0.2		0.3		0.4	
	M	m	M	m	M	m	M	m	M	m
Yarkoe 6	83.0	3.0	33.3	1.5	26.6	0.7	16.6	0.8	13.3	0.2
Shortandinskoe 7	100.0	5.0	26.6	2.0	13.3	0.9	6.6	0.6	0.0	0.0
K-10343	100.0	2.0	43.3	1.8	26.6	1.0	13.3	0.7	6.6	0.0
K-3742	66.6	4.0	30.0	2.0	13.3	0.3	0.0	0.0	0.0	0.0
Saratovskoe 6	43.3	2.0	23.3	1.1	13.3	0.5	6.6	0.1	0.0	0.0

Table 2. Efficiency of mutagen on the growth parameters of proso millet, mm

Genotypes	Treatments, %									
	0		0.1		0.2		0.3		0.4	
	M	m	M	m	M	m	M	m	M	m
Yarkoe 6	6.5	0.4	2.1	0.1	0.3	0.0	0.0	0.0	1.2	0.0
Shortandinskoe 7	12.2	0.5	8.0	0.1	5.4	0.2	3.7	0.1	0.0	0.0
K-10343	4.7	0.1	3.1	0.0	2.1	0.0	1.1	0.0	0.8	0.0
K-3742	10.2	0.3	7.9	0.2	5.2	0.2	4.7	0.1	0.0	0.0
Saratovskoe 6	10.1	0.2	7.2	0.3	5.2	0.1	3.5	0.1	0.0	0.0

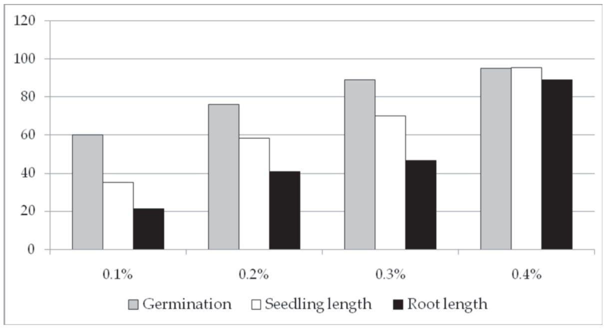

Fig. 1. Influence of various concentrations of sodium azide onto growth parameters of proso millet seedlings concerning control, (%)

10th day after sowing. The emergence of seedlings on the variants of the experiment was uneven. According to the results of field data, it was found that the percentage of germination in control samples on average ranged from 20 to 50%. The smallest germination rate was observed at 0.4% concentration, for example, in the variety Yarkoe 6 the germination rate was 3%, in the sample K-10343 - 1%, in other genotypes the germination rate was zero (Table 4).

Compared to the control, the highest germination ability at a mutagen concentration of 0.1% was observed in the varieties Yarkoye 6 and Saratovskoye 6, 7 and 13% higher than the control, respectively. In Shortandinskoye variety percentage germination increased from 44% in control 50% at 0.1% sodium azide and then decreased with increase in treatment. Also, the variety Shortandinskoye 10 (26%) and K-3742 (16%) samples were characterized by a good indicator of plant survival at a minimum concentration of sodium azide. After full ripening of the plants, the yield structure was analyzed for 25 proso millet plants isolated from a sheaf sample from reference sites (Table 5).

Selected plants from the collection nursery were analyzed according to morphological characteristics, panicle productivity, weight of 1000 grains, plant height, panicle length. The results in Table 5 show the effects of different concentrations of sodium azide on agronomic traits of proso millet. Panicle length, mass of seeds from a panicle and number of seeds per panicle decreased with treatment sodium azide in all genotypes. Number of seeds per panicle in the control was highest and decreased for 2-3 times with treatment sodium azide at 0.4% in all studied samples. In the K–10343 sample 1000 seed weight increase with increase in concentration and 0.3% had highest 4.3g was while control had the lowest with 3.3g.

Conclusion

Analysis of the obtained data demonstrated the inhibiting effectivness of sodium azide on all morphometric traits. Sodium azide affected most agronomic

Table 3. Effectiveness of sodium azide on root growth of millet roots, mm

Genotypes	Treatments, %									
	0		0.1		0.2		0.3		0.4	
	M	m	M	m	M	m	M	m	M	m
Yarkoe 6	4.2	0.2	3.5	0.3	3.2	0.1	3.2	0.2	1.0	0.0
Shortandinskoe 7	4.1	0.2	3.2	0.2	3.0	0.1	2.9	0.1	1.2	0.0
K-10343	3.9	0.1	3.1	0.0	2.8	0.0	2.6	0.0	0.0	0.0
K-3742	3.8	0.2	2.9	0.1	0.6	0.0	0.0	0.0	0.0	0.0
Saratovskoe 6	4.0	0.1	3.0	0.0	2.2	0.0	2.0	0.1	0.0	0.0

Table 4. Field germination of proso millet plants, %

Genotypes	Treatments, %				
	0	0.1	0.2	0.3	0.4
Yarkoe 6	28	30	18	2	3
Shortandinskoe 7	40	26	2	1	0
K-10343	30	4	4	4	1
K-3742	30	16	0	0	0
Saratovskoe 6	44	50	2	0	0

Table 5. Effect of NaN3 on the agronomic traits of the proso millet

Agronomic traits	0	0.1	0.2	0.3	0.4
		Yarkoe 6			
Plant business, pcs	1.3	1.2	1.0	1.0	0
Plant height, cm	60	58	38	35	0
Panicle length, cm	22	21	17	15	0
Number of seeds per panicle, pcs	663	450	276	107	0
Mass of seeds from a panicle, pcs	3.1	2.03	1.1	0.4	0
1000 seed weigh, g	5.2	4.8	4.3	4.1	0
		Shortandinskoe 7			
Plant business, pcs	1.3	1.1	1.0	1.0	0
Plant height, cm	55	55	45	33	0
Panicle length, cm	21	21	19	17	0
Number of seeds per panicle, pcs	430	440	270	230	0
Mass of seeds from a panicle, pcs	3.0	3.0	2.0	1.9	0
1000seed weigh, g	5.5	5.4	4.2	4.2	0
		K - 10343			
Plant business, pcs	1.3	1.1	1.1	1.1	1.1
Plant height, cm	55	61	60	60	62
Panicle length, cm	19	22	20	20	18
Number of seeds per panicle, pcs	1205	892	789	632	517
Mass of seeds from a panicle, pcs	3.9	2.9	2.1	2.7	3.7
1000 seed weigh, g	3.3	3.3	2.7	4.3	1.9
		K-3742			
Plant business, pcs	1.4	1.2	0	0	0
Plant height, cm	62	66	0	0	0
Panicle length, cm	19	20	0	0	0
Number of seeds per panicle, pcs	130	88	0	0	0
Mass of seeds from a panicle, pcs	1.0	0.7	0	0	0
1000 seed weigh, g	3.1	3.1	0	0	0
		Saratovskoe 6			
Plant business, pcs	1.1	1.2	1.2	0	0
Plant height, cm	39	46	45	0	0
Panicle length, cm	26	23	17	0	0
Number of seeds per panicle, pcs	459	420	160	0	0
Mass of seeds from a panicle, pcs	2.8	2.3	0.8	0	0
1000 seed weigh, g	5.6	5.2	4.7	0	0

traits such as germination, plant height, panicle length, panicle weigth and one thousand seed weight of proso millet in the field conditions. The results from this study also indicated that sodium azide was most effective at lower concentration.

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