Effect of drought on concentration of polyamines of new mutant lines of spring wheat

Wheat (Triticum aestivum L.) is grown in many countries around the world, its productivity is hampered due to a variety of abiotic stresses such as drought, salinity, and heat, particularly in arid and semiarid regions of the world. Of these, drought as complicated factor, is the major abiotic stress that is regarded as highly deleterious to growth and productivity of the wheat. Drought inhibits a variety of physiological processes in plants. Drought continues to be an important challenge to agricultural researchers and plant breeders. Drought is a major abiotic factor limiting the crops yield grown more than one third of the arable land around the world. Polyamines are endogenous plant growth promoters that affect a variety of physiological and metabolic functions, and they particularly involved in the flowering process. Studies conducted with other crop plants indicated that there is relationship between changes in polyamine content and drought tolerance. However, no information exists regarding to polyamine content of wheat under conditions of limited water supply. This study was aimed quantifying the effect of drought on different classes of polyamine such as putrescine, spermidine and spermine concentration in the parent line Zhenis and M, mutant lines developed on its genetic basis using gamma radiation doses of 100 and 200 Gy resulting changes in their concentrations. The article presents screening parent variety of spring wheat Zhenis and M, mutant lines developed on its genetic basis using gamma radiation doses of 100 and 200 Gy on polyamine content in plants grown under watered and drought condition. The wheat mutant germplasm, grown under irrigation had 4 samples, which are characterized by significantly increase in polyamines concentration. On the genetic basis of varieties Zhenis gamma radiation dose of 100 Gy on the basis of putrescine concentration have been identified two significant positive mutant lines under drought conditions.

Key words: drought, polyamines, putrescine, spermidine, spermine, wheat, mutant lines.

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Introduction

Wheat production is threatened by abiotic stresses prevailing in wheat agricultural systems. The most important abiotic stresses affecting wheat production include drought, heat, cold and salt stresses. Global climate changes are predicted to further increase causing further crop vulnerability. Drought is one of the most common environmental stresses that affect growth and development of plants. Drought continues to be an important challenge to agricultural researchers and plant breeders [1].

Many strategies are adapted by plants as a resort to abiotic environmental stresses. These adaptive mechanisms include changes in physiological and biochemical processes. Among them the accumulation of polyamines in plant tissue has drawn much attention. The polyamines, mainly putrescine (Put, a diamine), spermidine (Spd, a triamine) and spermine (Spm, a tetramine), are polycationic compounds of low molecular weight which could accumulate under a variety of abiotic stress conditions [2].

Polyamines are low-molecular-weight organic polycations with two or more primary amino groups-NH₂ and as shown they are present in bacteria, plants and animals. In plants, the diamine putrescine (PUT) and its derivatives, the triamine spermidine and the tetramine spermine are the most common polyamines and they have been reported to be implicated in a variety of plant metabolic and physiological functions [3]. Additionally, polyamines play a significant role in flower induction [4] along with flower initiation [5], pollination [6], fruit growth and ripening [7]. Research in other crops has indicated that changes in polyamines concentrations are a common plant response to a variety of abiotic stresses, including drought, salinity, high or low temperatures, and as well as biotic stresses [4].

Wheat (Triticum aestivum L.) is grown in many countries around the world, its productivity is hampered due to a variety of abiotic stresses such as drought, salinity, and heat, particularly in arid and semiarid regions of the world [8]. Of these, drought as complicated factor, is the major abiotic stress that is regarded as highly deleterious to growth and productivity of the wheat. Drought
inhibits a variety of physiological processes in plants, and its most significant negative effect can be observed on photosynthesis [9-10].

Water stress is the major factor limiting crop production worldwide. Water stress is one of the main integral components of drought including high temperature and high intensity of solar radiation. It alters a series of physiological, biochemical, and molecular responses [11]. Enhancing plant drought tolerance is the major challenge faced by modern agriculture, where polyamines can play an important role [12]. Plant polyamines are associated with the response of plants to diverse environmental stresses [12-14]. An increase in the endogenous polyamine content was reported in water-stressed plants confirming stress-specific roles of polyamines. Moreover, those with a higher number of amino groups such as spermidine (Spd) and spermine (Spm) were more effective in scavenging of reactive oxygen species, than putrescine [15-17]. In addition, the fluctuations of polyamine levels varied among plant genotypes. A significant increase in the free spermidine and spermine levels were observed in a drought-tolerant cultivar compared to increase of free putrescine levels in a drought-sensitive cultivar [18-19]. Furthermore, changes in the biosynthesis and catabolism of polyamines have occurred in plants under stress environments [13, 15, 19-21]. Decreased protein content and enhanced protein carbonyl formation have been also detected in stressed plants [22].

The objectives of our study were to determine the alterations caused by water-deficit on the polyamines content by their evaluation in wheat seedlings grown at watered and drought conditions in the parent line Zhenis and M lines of 100 Gy- and 200 Gy-dosed treatments developed on its genetic basis using these gamma radiation doses.

Materials and methods

The experiments were carried out in the year 2016 at the University of New-Hampshire, USA, Durham. The plant material used in the study were 10 M mutant lines of spring wheat which were developed using by irradiation treatment (100 and 200 Gy) on genetic base of cultivar Zhenis (the parent line)[23]. For screening response to drought the parent line and mutant germplasm were planted in pots in a in the tissue culture room. The seeds were sown in pots containing soil (SunGro, Professional Growing Mix). Ingredients of which were Canadian Sphagnum peat moss, bark, vermiculite, perlite dolomite, lime and wetting agent. In water conditions plants were irrigated daily for 14 days. In drought conditions 7 days seedlings were grown. The germination was carried out under tissue culture room conditions. Samples of leaves of 14-day old wheat seedlings grown under watered and drought conditions were collected for polyamines analysis.

Dansyl chloride and all polyamine and acetylpolyamine standards were purchased from Sigma (St. Louis, MO, U.S.A.). Acetone, toluene (Photrex grade), HPLC-grade methanol and acetonitrile were supplied by J.T. Baker (Phillipsburg, NJ, U.S.A.).

**Extraction of polyamines and their dansylation**

The polyamines were extracted from leaves three times by following freezing and thawing at room temperature. The polyamines extracts then were dansylated by following modifications of the procedure described by Smith and Davies [24]. Stock solutions of the various compounds were made in 5% perchloric acid and diluted to obtain the necessary final concentrations. Aliquots (50 l) of each solution or the centrifuged tissue extract were placed in 1.0-ml Reactivials (Pierce, Rockford, IL, U.S.A.) containing 100 mlk of a saturated sodium carbonate solution. A 100 mlk volume of dansyl chloride solution in acetone (10 mg/ml) was added to each vial. The vials were capped tightly and incubated in the dark in a water bath at 60°C for 1 h. A 50 mlk volume of proline (100 mg/ml) was added to the reaction mixture to remove excess dansyl chloride. After an additional 30 min incubation, acetone was evaporated from each vial by spinning under vacuum for 2 min in SpeedVac Evaporator (Savant, Farmingdale, NY, U.S.A.). A 400 mlk volume of toluene was then added to the solution and each vial was vortex-mixed for 30 s. The vials were centrifuged at 500 g for 2 min. After the aqueous and organic phases had separated, 200 mlk of the toluene layer were transferred to an Eppendorf tube. Toluene was completely evaporated in the SpeedVac and the residued dissolved in 1 ml of methanol or acetonitrile.

**Analysis of polyamines by high performance liquid chromatography (HPLC)**

The liquid chromatographic system (HPLC) consisted of a Perkin-Elmer series 400pump, a rhodyne injector valve fitted with a 6mk1 loop, a Perkin-Elmer Pecosphere-3x 3 CR Cls, 33 x 4.6 mm I.D. cartridge column (3 pm particle size), and a fluorescence detector (LS-1, Perkin-Elmer).
excitation and emission wavelength were set at 340 and 510 nm, respectively. Peak areas were calculated using a LC1-100 integrator (Perkin-Elmer).

A helpful hint: When heptanes sulfonate solution was left in the bottle for several days, the growth of microorganisms (not identified) in the connecting tubes caused problems with chromatography. The inclusion of 10% (v/v) acetonitrile in the heptanesulfonate solution eliminated this problem. Appropriate adjustments should be made in the gradient profile to achieve the desired concentrations at each step [25].

Results and discussion

In our study 4 lines of 100-Gy dosed of spring wheat 5(4), 6(5), 30(1) and 45(3) M7 mutant lines and the parent variety Zhenis were studied.

Putrescine concentration in leaves of parent variety Zhenis spring wheat seedlings grown under watered condition was 6.56±5.5 pmol/g fresh weight (Figure 1, A). In leaves of 100-Gy dosed M7 mutant lines of seedlings grown under watered condition varied in the range of 6.93 to 20.29 pmol/g fresh weight with a mean of 13.21 ± 5.61 pmol/g fresh weight for 4 lines (Figure 1, B).

At drought treatment, concentration of this polyamine in the parent variety Zhenis increased by 1.53 times.

In M7 mutant lines were found that putrescine concentrations significantly varied from 9.88 to 42.65 pmol/g fresh weight with a mean of 22.81 ± 13.97 pmol/g fresh weight for 4 lines (Figure 1, B). Genotypic response regarding to putrescine concentration in leaves under drought decreased in 5(4), 6(5) lines by about 0.90, or significantly increased by 2.86–2.98 times in 30(1) and 45(3) M7 mutant lines.

Thus, under drought, significant increase (about 3 times) in putrescine concentration were identified in leaves of two mutant lines 30(1) and 45(3) M7 developed by the genetic basis of cv. the parent variety Zhenis and 100 Gy dose gamma radiation.

In our study we determined drought induced responses in spermidine concentration in leaves of the parent variety of spring wheat Zhenis and M7 mutant lines (Figure 2). Under watered treatment in the parent line Zhenis spermidine concentration was 15.46±8.5 pmol/g fresh weight. At the same conditions of growth spermidine concentration in 100 Gy-dose M7 mutant lines of spring wheat ranged from 38.23 to 86.90 weight with a mean of 60.01 ± 20.38 pmol/g fresh weight for 4 lines (Figure 2, A).

Drought treatment induced great decrease in the spermidine concentration the parent variety of spring wheat Zhenis by 5 times with its value of 15.46 pmol/g fresh weight (Figure 2, B). In leaves of M7 mutant lines drought also caused a decrease in the spermidine concentration, however degree of this decrease was lower compared with from 0.31 to 0.64 times with a mean of 0.47 for M7 mutant lines. Genetic variations in the spermidine concentrations were in the range of 19.41 to 34.27 pmol/g fresh weight with a mean of 26.57 ± 6.9 pmol/g fresh weight for 4 these lines.

![Figure 1](image1.png)

**Figure 1** – Drought induced changes in putrescine concentrations in cv. the parent variety Zhenis and M7 mutant lines developed by its genetic basis and 100 Gy dose gamma radiation. (A) – watered and (B) drought conditions
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Figure 2 – Drought induced changes in the spermidine concentration in cv. the parent variety Zhenis and M7 mutant lines developed by its genetic basis and 100 Gy dose gamma radiation. (A) – watered and (B) drought conditions

Figure 3 shows genetic variations in another class of polyamines tested such as spermine in our study in cv. Zhenis and M7 mutant lines obtained on its genetic basis.

Spermine concentration in leaves of cv. cv. Zhenis at watered conditions of growth was determined as 79.61 ± 11.2 pmoles/g fresh weight (Figure 3, A).

Figure 3 – Drought induced changes in spermine concentration in cv. the parent variety Zhenis and M7 mutant lines developed by its genetic basis and 100 Gy dose gamma radiation. (A) – watered and (B) drought conditions

In M7, mutant lines spermine concentration varied in the range of 84.59 to 100.09 pmoles/g fresh weight with a mean of 92.42 ± 6.55 pmoles/g fresh weight for 4 lines (Figure 3, A). As indicate the results obtained, difference in spermine concentration at watered conditions between the parent line and mutant lines low. At drought, in cv. Zhenis spermine concentration almost was the same as it was found in watered leaves with a mean of 79.61 ± 11.2 pmoles/g fresh weight (Figure 3, B).

Very interesting response in relation to the spermine concentrations were observed in M7 mutant lines grown under drought (Figure 3, B). Some lines
such 5(4) and 6(5) showed the same response as the parent line, namely no drought–induced its changes. The lines 30(1)and 45(3) significantly increased spermine concentration in leaves by 17.56 and 9.84 times, with a mean of 30.71 ± 20.3 pmoles/g fresh weight for 5 lines (Figure 3, B).

**Figure 4** – Scatter plots of data for polyamines parameters, such as A – putrescine vs spermidine, B – putrescine vs spermine, C – spermine vs spermidine in the parent line Zhenis and M7 mutant lines developed by its genetic basis and 100 Gy dose gamma radiation at watered and drought conditions.
Thus, among three classes of polyamines such as putrescine, spermidine and spermine, determined in our research spermine shows the most pronounced response induced by drought in M7 mutant lines. Based on literature data we could assume its leading role related to drought resistance and the likelihood that the gamma radiation used to develop M7 mutant lines caused certain changes in the responsible for its synthesis genes.

To compare three classes of polyamines together one class to another Scatter plots of data for polyamines parameters were used; the example plots are shown in Figure 4 A, B and C. These plots demonstrate the spread of values and a fitted correlation line to test if there was any evidence for a relationship between the parameters. These data showed that correlations between each class of polyamines (putrescine vs spermidine, putrescine vs spermine, spermine vs spermidine) under watered conditions has a proportional relations (Figure 4). As it can be seen from Figure 4 drought treatment had produced effect to change of this relation in the opposite one. When these relations of each class of polyamines were compared with the parent a much higher level of reduction was observed.

**Conclusion**

Polyamines (PAs) are small, positively charged, organic molecules that are ubiquitous in all living organisms. The three common PAs in plants are putrescine (Put), spermidine (Spd) and Spm, with some plants also having thermospermine (tSpm) in place of or in addition to Spm. It is not surprising that fluctuations in their cellular contents are often related to varied responses of plants to different forms of stress and to different phases of growth activity. As much as their cellular functions are diverse, and sometimes contradictory, so are their roles in plant stress. They have been deemed important in preparing the plant for stress tolerance and to directly aid in ameliorating the causes of stress, and at the same time, their own catabolic products are responsible for causing stress damage. Several aspects of the relationship between PAs and abiotic stress in plants and their seemingly contradictory roles in the process have been reviewed over the years [26-28].

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