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Differences in salt tolerance between diploid and autotetraploid lines of *Lolium multiflorum* at the germination and vegetative stages

Olakunle Kelvin AKINROLUYO[1], Ieva URBANAVIČIŪTĖ[2], Kristina JAŠKŪNĖ[1], Vilma KEMEŠYTĖ[1], Gražina STATKEVIČIŪTĖ[1]

[1]Lithuanian Research Centre for Agriculture and Forestry, Institute of Agriculture
Instituto 1, Akademija, Kėdainiai distr., Lithuania
E-mail: kelvin.akinroluyo@lammc.lt

[2]Lithuanian Research Centre for Agriculture and Forestry, Institute of Horticulture
Kauno 30, Babtai, Lithuania

Abstract

Soil salinity is a global challenge emanating from climatic changes, depletion of fresh water reserves and extensive irrigation practices among other factors. Soil salinization still remains a huge concern in the realization of sustainable agricultural production. While emphasis has been placed on the food crops, forage production, which is an important component of the food chain, is affected as well.

The aim of this study was to evaluate the morphological and physiological response to salinity stress in diploid cultivars and auto-induced tetraploid lines of annual ryegrass (*Lolium multiflorum* spp. *multiflorum*). Diploid seeds and their induced tetraploid counterparts were germinated on filter paper moistened with different concentrations of sodium chloride (NaCl) solutions, and seedlings were treated with 500 mM NaCl for 10 days in controlled conditions. The effect of different salt concentrations on germination and seedlings was studied. Results showed that seeds from the induced tetraploid lines despite being bigger had higher germination index and lower median germination time (T50) values compared to the diploid progenitors. At the seedling stage, increase in the ploidy level had a role in conferring improved tolerance to salinity stress. The induced tetraploid lines had an advantage over their diploid counterparts as the induced tetraploid lines had significant reduction in their growth in response to salinity stress, higher relative water content and antioxidant activities.

Key words: abiotic stress, annual ryegrass, antioxidant activity, ploidy level.

Introduction

Environmental stresses affect the productivity of many agricultural crops globally. The world population is constantly increasing and is estimated to reach 9 billion people by the year 2050; however, the land acreage is fairly constant, therefore there is a need to explore lands that have not been used for agricultural purposes such as semi-arid regions and forests and high salinized soils to improve food production globally (Godfray et al., 2010).

Drought and salinity are one of the two main abiotic stresses that affect plant productivity. Recently, the threat posed by global warming is gradually becoming a reality as extended periods of high temperatures were recorded in the northern parts of Europe in 2018 (UNCC, 2019). Also, more than 6% of the global land area has severe salinity problem and this translates to about 800 million hectares (Yadav et al., 2011). In addition, extreme drought requires irrigation farming practices, which ultimately can result in increased soil salinity over a period of time (Qadir et al., 2014). A good approach to food sustainability is to increase the crop productivity in these suboptimal soil conditions, especially in high salinized soils.

High soil salinity affects the uptake of water from the roots and also cell growth and metabolism in the roots. Also, high saline conditions in the soil often lead to structural defects, high root zone pH, oxygen deficiency, impaired root respiration as well as nutritional imbalances (Roy et al., 2014). Also, studies have shown that plants grown on high saline substrates express salt-specific stress, oxidative stress and osmotic and ionic stress (Muscolo et al., 2013). The ionic stress is manifested by the accumulation of sodium and chloride ions in the plant tissues. The uptake of these ions causes

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severe ionic imbalance as the high concentration of sodium ions inhibits the uptake of potassium ions (Assaha et al., 2017). The unavailability of potassium ions results in physiological impairment in growth and development of the plants and can result in plant death. High soil salinity also often results in hyperosmotic stress, where there is a progressive loss of water from the leaves, while the absorption of water by the roots is significantly reduced (Tang et al., 2015). This hyperosmotic stress triggers physiological changes that are detrimental to the development of the plant including a reduction in photosynthetic activities and an increase in the reactive oxygen species (ROS) produced (Acosta-Motos et al., 2017). The ROS emanating from oxidative stress caused by high salinity substrate damages the cellular components and disrupts important cellular functions.

Annual ryegrass (*Lolium multiflorum* spp. *multiflorum*) is an important forage grass species from the family Poaceae and is widely cultivated in temperate regions. It is also widely used as a catch crop and is efficient in preventing soil erosion (Humphreys et al., 2010). It occurs naturally as diploid (2n = 2x = 14). Chromosome duplication has been achieved in many plant species and has been found to increase their tolerance to environmental stresses compared to their diploid counterparts (Sattler et al., 2016), while other studies reported diploids to have superior tolerance to abiotic stresses (Helgadóttir et al., 2018). However, little is known on the tolerance to salinity stress between diploid and tetraploid lines of annual ryegrass.

The aim of this study is to evaluate the role that ploidy has in the tolerance to salinity stress in annual ryegrass. Tetraploid lines were induced from diploid cultivars to maintain their genetic homogeneity and thus reducing the effect of genetic differences. The physiological response and the antioxidant activity changes to salinity stress were compared between diploid cultivars and auto-tetraploid lines.

Materials and methods

The research was conducted in 2019 at Laboratory of Genetics and Physiology, Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry.

Plant material and chromosome doubling. Tetraploid lines were induced from 8 diploid cultivars (Table 1) of annual ryegrass as described in Akinroluyo et al. (2018). The induced tetraploid lines were grown to maturity; seeds were collected and sown for the second generation. The ploidy level was also evaluated for the second generation of induced tetraploid lines; seeds of true tetraploid plants were collected and used in this study. In addition to the induced tetraploids, tetraploid cultivars 'Peleton', 'Caremo' and 'Wesley' were also used in this study.

Seed germination. Fifteen seeds of each cultivar and induced tetraploid line were placed on three layers of filter paper in a Petri dish in three replicates. The filter paper was moistened with either distilled water or different concentrations of sodium chloride (NaCl) solution. The salinity concentrations ranged from 120 to 200 mM. The germination was recorded daily for 10 days. The experiment was repeated three times. The germination percentage (GP), germination index (GI), mean germination time (MGT) and the median germination time (T50), which is the time to reach 50% of the germination in all the seeds, values were calculated as described in Coolbear et al. (1984) and Kader (2005) and was modified by Farooq et al. (2005):

Table 1. List of cultivars and induced tetraploid lines of *Lolium multiflorum* spp. *multiflorum* used in this study

Cultivar	Ploidy	Origin	Name of induced tetraploid
Druva	2×	Latvia	Druva-4x
Varpė	2×	Lithuania	Varpė-4x
Magloire	2×	France	Magloire-4x
Prompt	2×	France	Prompt-4x
Top speed	2×	France	Top speed-4x
Surrey nova	2×	USA	Surrey nova-4x
Grazer	2×	Germany	Grazer-4x
Shoot	2×	Denmark	Shoot-4x
Peleton	4×	Denmark	
Wesley	4×	Denmark	
Caremo	4×	Denmark	

$$GP = 100\ (x / n),$$

where x is the total number of germinated seeds, n – the total number of seeds;

$$GI = (10 \times n1) + (9 \times n2) + \cdots + (1 \times n10),$$

where n1, n2 ... n10 represents the germinated seed on the first, second and subsequently till he 10th day; 10, 9 ... 1 are weights given to the number of germinated seeds on the first, second and subsequent days till the 10th day, respectively;

$$MGT = \sum nt / \sum n,$$

where t (days) represents the time from the beginning of germination test, n – the number of germinated seeds at time t;

$$T50 = \frac{(ti + [\{(N / 2) - ni\}\ (ti - tj)]\ )/(ni - nj)}{ni - nj},$$

where N represents the final number of germination, n_i and n_j are cumulative number of seeds germinated by adjacent counts at times t_i and t_j when $n_i < N/2 < n_j$.

Effect of salt stress on annual ryegrass seedlings. Seeds from diploid, tetraploid and induced tetraploid lines and cultivars of annual ryegrass were germinated on 50:50 perlite:vermiculite mix substrate (vol.) in round plastic pots (diameter 9 cm, height 8 cm). The plantlets were allowed to develop for three weeks at 25 ± 2°C with a 16/8 h light/dark photoperiod before inducing the salinity stress. The plantlets were treated with 500 mM NaCl for 10 days. New unfolding leaves were marked at the nodes on the first day in five different plants in separate pots, and the leaf elongation was recorded on a daily basis at exactly the same time. The treatment was done in three replicates.

Relative water content (RWC) measurement. Plant water status at the end of the stress was expressed in terms of the RWC. Fresh leaf samples were cut and weighed immediately to determine the fresh weight (FW). The leaves were then placed in plastic bag containing water and left for 6 hours to reach the turgid weight

(TW). Then the leaves were blotted dry and the turgid weight was determined. Finally the leaves were placed in an oven at 70°C for 48 hours to determine the dry weight (DW). The relative water content was calculated using the formula (Smart, Bingham, 1974): RWC% = 100[(FW − DW) / (TW − DW)].

Antioxidant activity measurement. The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity was measured by slightly modifying the method described by Brand-Williams et al. (1995). The plant material was prepared by drying, homogenizing, suspending 0.5 g of stressed leaf samples and their respective control in 70% methanol, followed by extraction in a Sonorex Digital 10 P ultrasonic bath (Bandelin Electronic GmbH & Co. KG, Germany) for 60 min at 50°C and 480 W. Two ml of DDPH solution in 70% v:v methanol was mixed with 2 µL leaf methanol extract. The reduction in absorbance at 515 nm was measured and expressed as Trolox equivalent (TE) antioxidant capacity.

Statistical analysis. The statistical analysis was carried out using software SAS, version 9.4 (SAS Institute Inc., USA). The analysis of variance (ANOVA) was carried out and the significant differences between the means were determined using the Duncan's multiple range test. Correlation analysis was also carried out to check for relationships among the traits.

Results

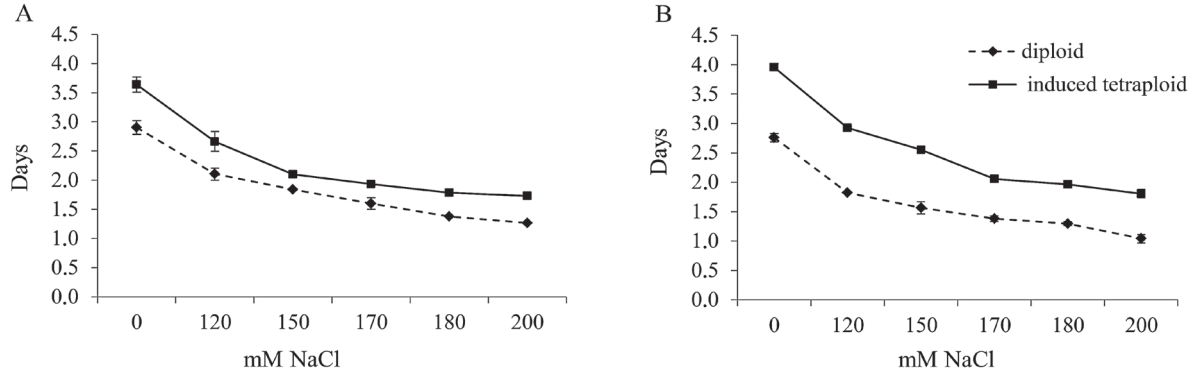
Genome duplication effect on germination of annual ryegrass under salt stress. Seeds from both cytotypes varied in length and weight. The induced tetraploid seeds were longer and heavier than their diploid progenitors (Table 2).

Table 2. The seed length and weight of diploid cultivars and respective induced tetraploid lines of *Lolium multiflorum* spp. *multiflorum*

Diploid cultivar / induced tetraploid line	Seed length mm	1000 seed weight g
Magloire	5.83 ± 0.17	2.77 ± 0.07
Magloire-4×	7.17 ± 0.17*	4.03 ± 0.05*
Druva	6.00 ± 0.00	2.39 ± 0.06
Druva-4×	7.00 ± 0.29*	4.23 ± 0.11*
Varpė	5.50 ± 0.00	2.81 ± 0.10
Varpė-4×	8.33 ± 0.61*	4.95 ± 0.06*
Grazer	5.00 ± 0.29	2.81 ± 0.08
Grazer-4×	6.50 ± 0.51*	4.60 ± 0.08*
Prompt	5.17 ± 0.17	2.70 ± 0.09
Prompt-4×	6.17 ± 0.17*	3.78 ± 0.05*
Shoot	5.67 ± 0.17	2.19 ± 0.08
Shoot-4×	7.33 ± 0.34*	4.08 ± 0.11*
Surrey nova	4.83 ± 0.17	2.93 ± 0.10
Surrey nova-4×	6.67 ± 0.17*	3.89 ± 0.08*

Note. Data shown as mean ± standard error of three replicates; the means followed by * between diploid cultivar and corresponding induced tetraploid line are significantly different at $p \leq 0.05$ (Duncan's multiple range test).

The effect of salinity stress on germination is shown in Figure 1 and Table 3. Salinity stress appears to delay the outset of germination or inhibit germination in both cytotypes and across the cultivars. Also, the inhibition of germination is increased as the salinity concentration increased ($r = 0.86$, $p \leq 0.01$).



Note. The error bars represent the standard error of the mean.

Figure 1. Germination index of diploid cultivars and respective induced tetraploid lines 'Magloire' (A) and 'Varpė' (B) of *Lolium multiflorum* spp. *multiflorum* in different NaCl concentrations

The germination percentage, mean germination time, germination index and T50 were calculated for both cytotypes. The result showed that overall germination parameters were reduced in the seeds of both diploid cultivars and the induced tetraploids when compared with their respective control; however, chromosome duplication appears to have a role in improving the tolerance to salinity stress at the germination stage as seen in the germination index and T50 values. The cultivar differences also contributed to the observed differential response to the salinity treatments.

Genome duplication effect on the seedling growth of annual ryegrass under high saline conditions. Seedlings of both diploid and tetraploid *Lolium multiflorum* spp. *multiflorum* (three weeks old) were grown in controlled conditions. New unfolding leaves were measured in this experiment. The leaf elongation was determined during stress treatment as well as in the control in non-destructive daily leaf length measurements. The leaf elongation was measured at the onset of the stress till the end of the experiment. The effect of high salinity treatment was apparent in both induced

Table 3. Germination percentage, mean germination time, germination index and median germination time (T50) of different diploid cultivars and the respective induced tetraploids of *Lolium multiflorum* spp. *multiflorum* after salinity (200 mM NaCl) treatment

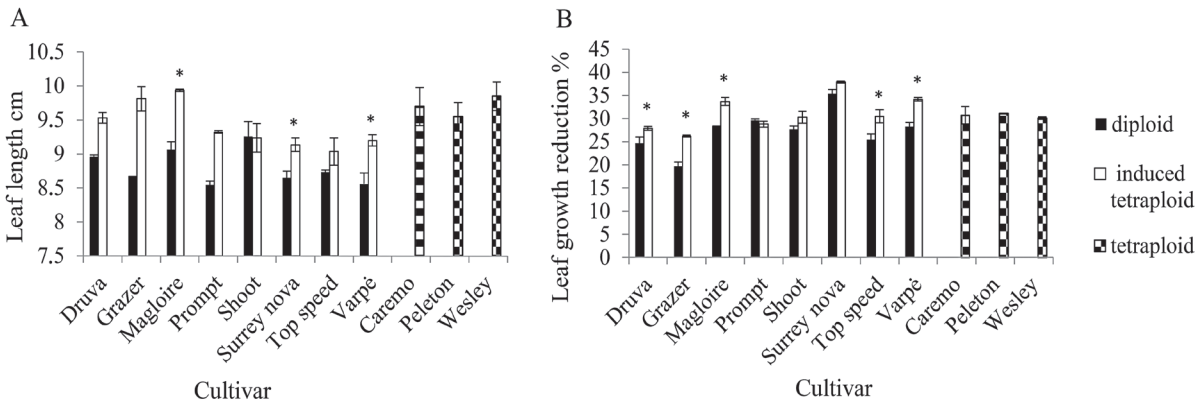
Diploid cultivar / induced tetraploid line	Germination %	Mean germination time, days	Germination index, days	Median germination time (T50), days
Magloire (control)	91.67 ± 2.33 abc	4.50 ± 0.00 fg	2.93 ± 0.22 cd	3.93 ± 0.07 fghi
Magloire-4× (control)	93.33 ± 0.00 abc	3.93 ± 0.07 h	3.62 ± 0.09 ab	3.45 ± 0.05 i
Magloire	63.33 ± 3.33 de	8.00 ± 0.09 bc	1.29 ± 0.04 gh	7.50 ± 0.00 ab
Magloire-4×	90.64 ± 3.33 abc	7.48 ± 0.05 c	1.87 ± 0.09 f	6.58 ± 0.25 c
Druva (control)	91.67 ± 2.33 abc	4.33 ± 0.00 gh	3.18 ± 0.38 bc	3.74 ± 0.01 ghi
Druva-4× (control)	98.00 ± 3.33 ab	4.38 ± 0.02 gh	3.37 ± 0.11 bc	3.85 ± 0.08 fghi
Druva	46.67 ± 0.00 ef	6.64 ± 0.07 d	1.10 ± 0.00 hi	7.58 ± 0.04 ab
Druva-4×	53.33 ± 0.00 ef	6.88 ± 0.13 d	1.18 ± 0.01 h	6.33 ± 0.04 cd
Varpė (control)	80.00 ± 0.00 bcd	4.75 ± 0.09 fg	2.56 ± 0.04 de	4.31 ± 0.06 efg
Varpė-4× (control)	100.00 ± 0.00 a	3.90 ± 0.04 h	3.88 ± 0.04 a	3.44 ± 0.02 i
Varpė	46.67 ± 6.66 ef	8.23 ± 0.11 ab	0.88 ± 0.14 hi	7.88 ± 0.38 ab
Varpė-4×	84.25 ± 3.47 abc	6.83 ± 0.08 d	1.71 ± 0.09 fg	6.25 ± 0.05 cd
Grazer (control)	82.30 ± 5.87 abc	5.35 ± 0.44 e	2.40 ± 0.10 e	4.58 ± 0.20 e
Grazer-4× (control)	95.67 ± 3.33 ab	4.93 ± 0.14 ef	3.01 ± 0.03 cd	4.43 ± 0.15 ef
Grazer	38.42 ± 2.34 f	7.88 ± 0.28 bc	0.70 ± 0.04 i	7.52 ± 0.15 ab
Grazer-4×	65.33 ± 2.34 de	6.73 ± 0.21 d	1.21 ± 0.03 h	6.24 ± 0.08 cd
Prompt (control)	98.00 ± 3.33 ab	4.25 ± 0.11 gh	3.53 ± 0.27 ab	3.64 ± 0.06 hi
Prompt-4× (control)	100.00 ± 0.00 a	4.50 ± 0.10f g	3.40 ± 0.06 bc	3.84 ± 0.09 fghi
Prompt	78.37 ± 5.87 cd	6.58 ± 0.18 d	1.81 ± 0.19 f	6.08 ± 0.42 cd
Prompt-4×	56.45 ± 5.93 e	8.04 ± 0.24 b	1.09 ± 0.23 hi	7.42 ± 0.09 ab

Note. Data shown as mean ± standard error of three replicates; the means followed by the same letter within each column are not significantly different ($p > 0.05$, Duncan's multiple range test).

tetraploids and the diploid cultivars showing a significant reduction in the leaf growth and shoot development. Our results showed that the induced tetraploids had longer leaf when compared to their parental diploids during the salinity treatment (Fig. 2A). Also, the induced tetraploids showed a higher reduction in their leaf elongation than their diploid progenitors when compared with their respective control experiment (Fig. 2B). Differences in the cultivar leaf length and leaf growth reduction were also observed.

Plants subjected to salinity stress showed signs of wilting when compared to the control. Salinity stress also reduced the relative water content. The relative water content varied across the cultivars and most of the induced tetraploids were found to have higher relative water content when compared to their diploid progenitors (Table 4). The differences observed between the means were significant.

Genome duplication effect on the antioxidant activities of annual ryegrass under salt stress. The antioxidant activities in response to the salinity stress were determined in both diploids and tetraploids. The result showed that the induced tetraploids produced more antioxidant activity response than their diploid progenitors, except for the cultivar 'Prompt'. A significant positive correlation was also found between the antioxidant activities and the germination index ($r = 0.55$, $p \leq 0.05$) and the reduction in leaf elongation during salinity stress ($r = 0.64$, $p \leq 0.05$).



Note. Data shown as mean ± standard error of three replicates; the means followed by * between diploids and corresponding induced tetraploids are significantly different at $p \leq 0.05$ (Duncan's multiple range test).

Figure 2. The leaf length (A) and leaf growth reduction (B) in seedlings of *Lolium multiflorum* spp. *multiflorum* treated with 500 mM NaCl

Table 4. The effect of salinity stress on the relative water content and the antioxidant activity response in diploid cultivars and the respective induced tetraploid lines of *Lolium multiflorum* spp. *multiflorum* seedlings

Diploid cultivar / induced tetraploid line	Relative water content %	Antioxidant activity (control) μmol TE g^{-1}	Antioxidant activity (500 mM NaCl) μmol TE g^{-1}
Varpė	68.76 ± 0.19 g	23.73 ± 3.22 de	34.97 ± 0.78 fg
Varpė-4×	75.87 ± 0.49 de	35.48 ± 1.34 a	47.26 ± 0.95 ab
Druva	80.18 ± 1.18 c	32.71 ± 1.01 ab	36.63 ± 1.35 g
Druva-4×	71.77 ± 0.5 f	29.55 ± 1.35 bc	44.26 ± 0.98 bc
Magloire	69.06 ± 0.31 g	33.42 ± 1.81 ab	41.51 ± 2.63 cd
Magloire-4×	80.64 ± 1.01 c	33.18 ± 1.15 ab	49.15 ± 0.11 a
Grazer	75.92 ± 1.43 de	22.97 ± 0.15 e	28.82 ± 2.28 h
Grazer-4×	74.37 ± 0.93 ef	24.73 ± 0.55 de	39.00 ± 1.97 de
Surrey nova	87.61 ± 1.02 a	27.34 ± 0.54 cde	32.84 ± 1.34 fg
Surrey nova-4×	83.50 ± 1.01 b	23.33 ± 2.87 de	42.70 ± 2.33 c
Prompt	73.67 ± 0.21 ef	27.82 ± 0.72 cd	36.52 ± 1.77 ef
Prompt-4×	81.00 ± 1.01 bc	28.14 ± 0.02 cd	38.84 ± 2.35 de
Top speed	77.36 ± 0.69 d	27.46 ± 0.50 cde	35.34 ± 1.45 efg
Top speed-4×	88.08 ± 0.63 a	24.01 ± 0.78 de	41.34 ± 1.23 cd
Shoot	74.02 ± 1.00 ef	30.02 ± 0.52 bc	35.66 ± 0.55 efg
Shoot-4×	80.42 ± 0.36 c	32.81 ± 1.14 ab	43.24 ± 0.89 c

Note. Data shown as mean ± standard error of three replicates; the means followed by the same letter within each column are not significantly different ($p > 0.05$, Duncan's multiple range test); TE – Trolox equivalent.

Discussion

The outset of the germination process begins with seed imbibition, in which water is absorbed by seeds to make the nutrients in the endosperm available. Imbibition is a critical stage in the germination process and has to be completed before germination occurs. The imbibition process depends on several factors, including the temperature, water, oxygen, permeability of the seed coat, seed size and osmotic potential (Louf et al., 2018). Several studies (Matthews, Khajeh-Hosseini, 2007; Ahmed et al., 2017) have shown that the germination and seedling growth are significantly affected by salt stress; however, little is known on the role that ploidy has in annual ryegrass. Studies have reported that imbibition is completed faster in the smaller seeds within the same ploidy level because of the increase in surface area to volume ratio, hence, increasing the absorption of water is leading to faster germination (Schneider, 1998; Souza, Fagundes, 2014). However, other studies reported that tetraploid seed germinated faster than their diploid counterparts (Eliášová, Münzbergová, 2014).

Our results showed that induced tetraploid seeds of annual ryegrass were bigger and heavier than their parental diploids and in most cases germinated faster than the diploid counterparts. This raises the question if increase in the ploidy level confers an advantage in seed germination and, even more, under stress condition.

The performance of seeds can be seen by comparing the germination parameters between the diploids and induced tetraploids. The mean germination time (MGT) for both cytotypes varied across the cultivars and induced lines and was clearly not accurate in explaining the role of ploidy in seed germination. Although some researchers have used the MGT to evaluate the seed vigour of many plants (Matthews et al., 2012; Chen et al., 2013), other studies found the MGT to be inaccurate arguing that seeds can have different final germination percentage and have the same MGT because seeds can germinate across a different spread (Kader, 2005). The MGT expressed better the day, in which most of the seeds germinated in a seed lot or accurately defined the mean lag period between the start of imbibition and germination for each seed (Matthews, Khajeh-Hosseini, 2007). On the other hand, the T50 gave a better understanding of the speed of germination (Soltani et al., 2015). Our results indicated that the induced tetraploids had lower T50 values, especially during the salinity stress, except for the cultivar 'Prompt'.

The germination index gives a more accurate measurement of the germination than the germination percentage and the mean germination time because it takes cognizance of the germination percentage, speed of germination and the spread of germination (Javaid et al., 2018). It is clearly seen from our results (Table 3) that the induced tetraploid seeds had a higher germination index than their diploid progenitors both in the control and under stress conditions, except for the cultivars 'Prompt' and 'Druva'. In diploid cultivar 'Prompt' the seeds had a higher germination index than the induced tetraploid counterpart only during salinity stress, while no significant difference was found in the germination index of diploid and induced tetraploid seeds of 'Druva' both in the control and under salinity stress.

Generally, the effect of salinity on seed germination occurs via the ionic toxicity, osmotic effect or the combination of both effects (Panuccio et al., 2014). Salinity often increases the osmotic potential while decreasing the water potential making water unavailable to plants. The lower the osmotic potential in seeds, the more seed can absorb water and complete imbibition. Zhang et al. (2010) further explained that seeds in saline conditions can have a decreased osmotic potential by excluding salt from the cells while using other organic solutes as osmolites to maintain the osmotic potential. Bigger seeds at a higher ploidy level are at an advantage here than smaller seeds at a lower ploidy level, as they

have more carbon reserves and can generate a lower osmotic potential and thus, alleviating the need to absorb sodium. Alternatively, seeds can accumulate and use sodium and chloride ions as osmolites while having a mechanism that neutralizes their toxic effect. However, it remains unclear what is the mechanism used by annual ryegrass in maintaining a water potential gradient during the salinity.

Our results showed a medium positive ($r = 0.55$) correlation between antioxidant activities during salinity stress at the seedling stage and the germination index. Interestingly, the increases in antioxidant activities during salinity stress at the seedling stage in the induced tetraploid lines were significant when compared to their parental diploid, except in the cultivar 'Prompt', which also had higher germination index compared to its corresponding induced tetraploid. While some studies have used salinity tolerance at the germination and seedling stages as an indicator for screening tolerant genotypes (Shahid et al., 2012; Ravelombola et al., 2017), other studies indicated the opposite suggesting that the tolerance to salinity stress might be specific for various developmental stages (Lauchli, Epstein, 1990). However, the relationship between the antioxidant activities at the seedling stage and the germination index alone cannot fully explain if the tolerance at the germination stage in *Lolium* could be an indicator of tolerance at other developmental stages. More studies involving physiological responses at various developmental stages are needed to understand how seeds reduce their osmotic potential in the germination process during salinity stress and also to determine an effective tolerance screening stage in *Lolium*.

At the vegetative stage, the first effect of salinity stress occurs in the root system of plants and this impairs the growth due to the osmotic stress. The osmotic stress reduces the availability of water to the plants and also generates reactive oxygen species (ROS) (Ashraf, Foolad, 2013). One of the first metabolic responses of plants under stress is the growth inhibition and down regulation of energy metabolism indicating that plants conserve energy (Cramer et al., 2011). The reduction in growth especially in the leaf area usually occurs by inhibiting protein synthesis. This is an avoidance mechanism that helps to reduce water loss via transpiration (Rodriguez et al., 2005). Our results show that while the induced tetraploids have longer leaves than their diploid counterparts, the induced tetraploids were able to slow down the leaf growth more than their diploid progenitors. Also, the induced tetraploid lines in most cases had higher relative water content when compared to their corresponding diploid progenitors. The response to salinity stress involves a cascade of reactions involving many genes at the molecular level; however, duplication of the genetic materials seems to have an advantage over the diploid in the first response to salinity in annual ryegrass.

Prolonged exposure to salinity stress leads to ion toxicity and nutrient imbalance. This often results in sodium toxicity and generation of ROS (Torre-González et al., 2017). Plants growing in optimal conditions are said to be redox homeostatic because there is equilibrium in the production and scavenging of ROS. When plants generate high levels of ROS with an inefficient mechanism in scavenging the ROS, it causes an imbalance in the cellular redox, hence, leads to oxidative stress (Sharma et al., 2012). Plants generally combat high levels of ROS by activating the enzymatic and non-enzymatic system that scavenges the ROS. The enzymatic system involves enzymes such as superoxide dismutase, peroxidase, catalase, glutathione reductase and ascorbate peroxidase (Huseynova et al., 2014). The non-enzymatic system involves tocopherols, glutathione and ascorbic acid, which are also involved in ROS detoxification (Caverzan et al., 2016).

The synthesis of the antioxidants and their activities are altered when plants are subjected to stress conditions. In addition, polyploids can increase their tolerance to stress by altering their physiology, phenology and morphology (Adams, Wendel, 2005). Reports have shown that the increase in stress tolerance correlates with the increase in antioxidant activities (Aghaei et al., 2009). Our results indicated that the induced tetraploid lines responded with significant higher antioxidant activities than their diploid progenitors, except for the cultivar 'Prompt'. This also agrees with the report from Meng et al. (2011), where increased antioxidant activities contributed to the tolerance of auto-induced tetraploid turnips over their diploid progenitors. Our results also showed a positive correlation between reduction in growth and the antioxidant activities suggesting that the tetraploid lines are better adapted in their first response to salinity stress.

Conclusions

1. Salinity stress significantly inhibits the germination of annual ryegrass (*Lolium multiflorum* spp. *multiflorum*) seeds. Chromosome duplication appears to have an important role during germination in saline conditions. The induced tetraploid lines in most cases had higher germination index and lower median germination time (T50) values than their diploid progenitors.

2. Polyploidy contributes to tolerance to salinity stress in annual ryegrass as observed in the morphological and physiological response at the vegetative stage. The induced tetraploid lines showed significant reduction in leaf growth and, hence, were superior in their first response to salinity stress than their diploid progenitors.

3. The induced tetraploid lines of annual ryegrass had higher antioxidant activities than their parental diploids indicating that polyploidy contributes to tolerance to salinity stress that often leads to oxidative damage in plant cells.

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Atsako į druskingumo stresą skirtumai *Lolium multiflorum* diploidinių bei tetraploidinių augalų dygimo ir vegetatyvinio augimo tarpsniais

O. K. Akinroluyo[1], I. Urbanavičiūtė[2], K. Jaškūnė[1], V. Kemešytė[1], G. Statkevičiūtė[1]

[1]Lietuvos agrarinių ir miškų mokslų centro Žemdirbystės institutas
[2]Lietuvos agrarinių ir miškų mokslų centro Sodininkystės ir daržininkystės institutas

Santrauka

Klimato kaita, gėlo vandens išteklių mažėjimas ir intensyvus drėkinimas yra vieni iš veiksnių, sukeliančių dirvožemių druskingumo didėjimą. Dėl druskėjimo procesų visame pasaulyje prarandami žemdirbystei tinkami plotai. Nors didžiausias dėmesys skiriamas maistinių augalų produkcijai, nukenčia ir pašarams skirti žolynai, kurie yra svarbi mitybos grandinės dalis.
Tyrimo tikslas – įvertinti gausiažiedės svidrės (*Lolium multiflorum* spp. *multiflorum*) diploidinių ir autotetraploidinių augalų morfologinį bei fiziologinį atsaką į druskingumo stresą dygimo ir augimo tarpsniais. Diploidinės ir indukuotos tetraploidinės sėklos buvo daigintos skirtingos koncentracijos druskos tirpaluose. Diploidinių ir tetraploidinių sėklų daigai 10 dienų kontroliuojamomis sąlygomis buvo veikiami 500 mM NaCl. Indukuotų tetraploidinių linijų augalų sėklos buvo didesnės ir pasižymėjo didesniais dygimo indekso bei vidutinio daigumo laiko (T50) įverčiais nei atitinkamos diploidinės tėvinės veislės. Vegetatyvinio augimo tarpsniu autotetraploidiniai augalai buvo atsparesni didesniam druskingumui, išsiskyrė didesniu santykiniu vandens kiekiu ir antioksidaciniu aktyvumu nei diploidinių veislių augalai.

Reikšminiai žodžiai: abiotinis stresas, antioksidacinis aktyvumas, gausiažiedė svidrė, ploidiškumas.