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IMPROVEMENT OF *TRIGONELLA MARITIMA* DELILEE X. POIR. GERMINATION BY POLYPLOIDIZATION

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Abstract

In this study, we determined the effect of polyploidization induced by colchicine on the germination power of *Trigonella maritima* seeds. Germinated seeds of diploid plants of *T. maritima* were immersed in 0.05% colchicine solution for 4 h and then sown in pots in natural conditions. The seedlings from these seeds were compared with those of untreated seeds. The colchicine significantly increased the DNA content in polyploid seeds. Polyploidy increases the size and weight of seeds. The germination % in polyploid seeds was 90.52% compared to only 41.67% in diploid seeds

Introduction

Trigonella maritima is a legume and used as fodder plant. The family of leguminous plants shows a high percentage of proteins and vitamins and a strong capacity to fix atmospheric nitrogen. Thus, the plants of this family are often used for the improvement of the pastures (Mbaye *et al*., 2002). Seed germination is most important transition stage for annual plants (Goldberg *et al*., 2001) and it determines the success of plant growth (Ungar & Badger, 1989; Khan & Rizvi, 1994). Germination indicates the whole of the processes, which are spread out from the beginning of the rehydration of seed to the emergence of the radicle out of the teguments. This phenomenon requires several favourable external conditions: optimum temperature, good oxygenation and enough water availability. In addition, seed must be ripe for germination, i. e. all its parts (tegument, reserve tissues and embryo) must be completely differentiated in their morphology, its germinative capacity must be preserved (this depends on the conditions of conservation and the longevity of seeds) and possible inhibitions must be raised (tegumentary or embryonic dormancy) (Gorenflot, 1983; Heller *et al*., 2000). Certain substances favour germination, such as Potassium Nitrate, Calcium salts, gibberellins, cytokinins. Others are, on the contrary, inhibitors of the germination such as coumarin which is a lactone, used in agriculture against the germination of weedy grasses. Coumarin is naturally found in Leguminous and Rubiaceae. Among the growth hormones, the abscisic acid (ABA), component metabolized under the action of Na^+, acts by limiting the absorption of water and by inhibiting the synthesis of specific enzymes of germination (Belkhodja & Bidai, 2004). Age of the seeds also influences germination. Indeed, oldest seeds have the highest rate of germination and the shortest average time of germination while young seeds show a low germinative capacity due to insufficient physiological maturity (Ouled Belgacem *et al*., 2004; Kettenring & Galatowitsch, 2007).

Several techniques have been used to improve and accelerate seed germination: mechanical (Magini, 1962; Mbaye *et al*., 2002) or chemical scarification (Côme, 1970; Mbaye *et al*., 2002) pre-treatment with the cold and moisture, one period follow-up at high temperature then of a new period at low temperature, this thermal shock is able to soften teguments (Mbaye *et al*., 2002).

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In this work, we evaluate the effect of the polyploidization on the germination of seeds of *Trigonella maritima* which have a weak germinative capacity and some of the techniques for germination improvement fails when applied to *T. maritima.*

Materials and Methods

Diploid seeds of *T. maritima* Delilee x. Poir. were collected from Monastir (East of Tunisia) during March 2006, from one population. These were germinated in distilled water at 25°C. After two days, teguments were removed and the seedlings were immersed in 0.05% colchicine solution for 4h and then abundantly rinsed with distilled water. The seedlings were planted in pots under the natural conditions. Duration of Sunlight was 16h and temperature ranged between 15°C and 26°C during the experimental period. The plants were followed till maturity; the seeds collected were polyploid. Floral buttons were collected to determine the caryotype and study the pollen grains (results not given). As per the chromosomal chart, the seeds of same branches are used to compare their germination capacity with the diploid seeds (DS). These seeds are indicated by polyploid seeds obtained by immersion (PSI). A second technique of polyploidization was to place cotton soaked with colchicine on the caulinary meristem (between the two cotyledons) for 24 h, four days after germination. During the exposure time, cotton was wetted to avoid its desiccation. Then meristem was abundantly rinsed to eliminate the colchicine traces. Polyploid seeds obtained by this technique are indicated as PSC (polyploidy seeds obtained by cotton application).

DNA of seeds from plants previously subjected to colchicine treatment was quantified to determine if they were polyploid. DNA content was determined according to Solymosy *et al*., (1968). We also compared the morphological characters of the seeds.

For each genotype, three replicates of 50 seeds were weighed and germinated on wet filter paper in Petri dishes at room temperature under natural light. The Petri dishes were placed in single completely randomized block design. The seed germination of each set was recorded up to 14 days. *T. maritima* seeds were treated with fungicides. The seeds were first immersed in Ethanol (70%) for 2 min and then washed with distilled water, then immersed in 50% hypochlorite sodium solution for 15 min, then rinsed 4 times in sterile distilled water. Considering the hardness of seed teguments, seeds were mechanically scarified with abrasive paper.

We determined the speed of germination (MDG) as per Lachiheb *et al*., (2004) as under:

$$MDG = T1 + [(0.5 – G1) / (G2 – G1)] * (T2 – T1)$$

MDG: Median duration of germination.

G1: Cumulated percentage of the germinated seeds to which the value is closest to 50% of the germinative capacity by lower value.

G2: Cumulated percentage of the germinated seeds to which the value is closest to 50% of the germinative capacity by higher value.

T1: Time necessary to reach G1.

T2: Time necessary to reach G2.

Data of 3 complete experiments were combined for analysis of variance and means were separated using Duncan's Multiple Range test at P = 0.05.

Results

Checking of polyploidy: To be sure of the ploidy level, observation of the pollen grains cells mother caryotype, taken from floral buttons anthers were carried out in a previous work (results not yet published). Seeds used in this work were collected from the same branches. We observed differences in the chromosomal load of the three seed populations. Indeed, the microscopic observation revealed different chromosomal charts. These results enabled us to consider that seeds used in this study are polyploid and that the differences recorded between the three types of seeds would be due to a different genic load. Also, we carried out a dosage of the DNA in the different seed types.

Spectrophotometric method: Table 1 presents the DNA content of the various seed types. The results show that polyploid seeds have higher DNA contents than diploid ones (DS). Indeed, we record a content of 284.86 µg/ml for the diploid seeds and of 539.39 and 567.85 µg/ml for PSC and PSI, respectively.

Moreover, the $DO_{(260)}/DO_{(280)}$ ratio was 2.15, 1.70 and 2.13 for the DS, PSI and PSC respectively, which indicates that the prepared solutions of DNA are more or less pure, this quantification was checked by the fluorescence method.

Fluorescence method: To confirm the preceding results and to compare the DNA content of the various seed types, we carried out the fluorescence method. By comparing the DNA profiles of the diploid and polyploidy seeds (Fig. 1), we notice that the DNA bands thickness is more significant for the polyploid seeds either PSI or PSC.

Dimensions seeds: Polyploidy influence significantly size of seeds. Length was 4.27 mm and 4.55 mm for diploid and polyploid seeds, respectively. In the same way the width testified an increase but not significant (Fig. 2 and Table2).

Mass seeds: The results appearing in table 2 show that polyploidy provokes a significant increase in the mass of individual seeds. The mass of the polyploid seeds was about 0.85mg, that of the diploids was only 0.68mg.

Polyploidization and germination: The percentage and the median duration of germination according to the type of seeds (DS, PSC and PSI) are presented in table 3. Polyploidy improved the percentage of germination. This improvement was more spectacular and significant at PSI seeds. Indeed, this percentage was 90.52% and 59.45% for the polyploid PSI and PSC respectively and 41.67% for the diploid seeds.

As for the speed of germination, expressed by the median duration of germination, the results show that the lowest value is recorded for the diploid seeds (2.82 days) and the highest value for those given by the plants treated with colchicine at the meristematic level (12.38 days). For seeds of type PSI the median duration of germination is 5.28 days (Table 3).

Thus the diploid seeds germinate more quickly, those polyploid have slowed down germination, this deceleration is more distinguishable for seeds coming from plants whose meristem was treated by the colchicine compared with those coming from plants whose seeds were immersed in a colchicine solution.

Table 1. Contents of DNA extracted from diploid seeds (DS) and those polyploid induced by immersion in the colchicine solution (0.05%) of germinated seeds (PSI) or by meristematic application of cotton soaked by the same solution (PSC), of *T maritima.*

Seeds	Contents of DNA (µg/ml)
DS	284,86a
PSC	539,39b
PSI	567,85c

The averages followed by the same letter in a column are not significantly different at $p<0.05$.

Table 2. Averages of mass (MS) (mg) and dimensions seed (length (l) and width (w) of diploids seeds (DS) and those polyploid induced by immersion in the solution of colchicine (PSI) of germinated seeds or by meristematic application of cotton soaked by the same solution (PSC), of *T. maritima.*

Seeds	MS (mg)	l (mm)	w (mm)
DS	0.68 ± 0.14a	4.27 ± 0.12a	3.19 ± 0.07a
PSC	0.75 ± 0.15ab	4.31 ± 0.11a	3.21 ± 0.10a
PSI	0.85 ± 0.13b	4.55 ± 0.09b	3.28 ± 0.12a

The averages followed by the same letter in a column are not significantly different in $p<0.05$.

Table 3. Effect of polyploidy on the percentage and the median duration of germination of diploid seeds (DS) and those polyploid induced by immersion in the colchicine solution (PSI) or by meristematic application of cotton soaked by the same solution (PSC), of *T. maritima.*

	DS	PSC	PSI
Germination percentage (%)	41.67a	59.45a	90.52c
Median duration of germination (days)	2.82a	12.38b	5.28c

The averages followed by the same letter in the same line are not significantly different in $p<0.05$.

The comparison of the germinative behaviour of the various types of seeds was also made while following their germinative kinetics (Fig. 3), which describes two phases for the various types of seeds: linear increasing of variable duration according to treatments, and a phase of stability which shows a plate where the total number of seeds able to germinate is reached. The results show that polyploidy caused an increase in the duration of the linear phase of the curve. Indeed, the maximum percentage of germination was reached after two days for the diploid seeds and after 11 days for the polyploid ones obtained by the two types of treatments. However, we note that the slope of the linear phase was much weaker for the polyploid that induced by meristematic imbibition (PSC) compared with those induced by immersion (PSI) (Fig. 3).

Discussion

Colchicine induced an increase in DNA content of seeds, also the microscopic observation revealed chromosomal charts different for the three types of seeds (results not yet published). These results enabled us to consider seeds used in this study as polyploid one and that the differences recorded between the three types of seeds would be due to a different genic load.

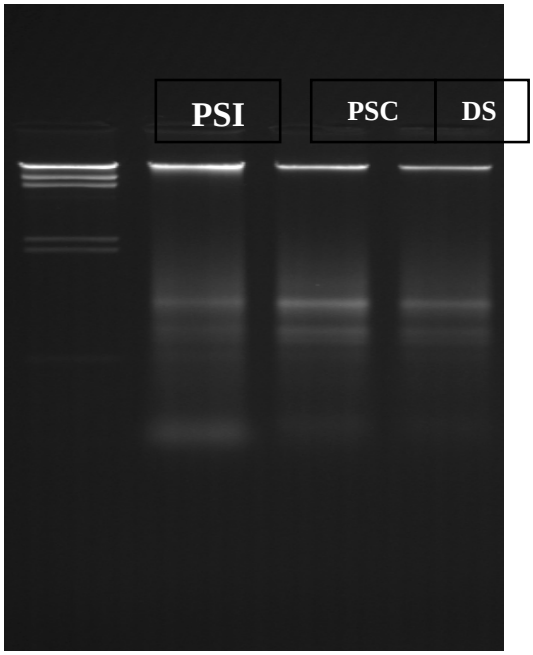

Fig. 1. Profile of migration of the DNA extracted from diploid seeds (DS) and those polyploid induced by meristematic application by cotton soaked (PSC) or by immersion in the colchicine solution of germinated seeds (PSI), of *T. maritima.*

Fig. 2. Diploid seeds (DS) and those polyploid induced by immersion in the solution of colchicine (PSI) of germinated seeds or by meristematic application of cotton soaked by the same solution (PSC), of *T. maritima.*

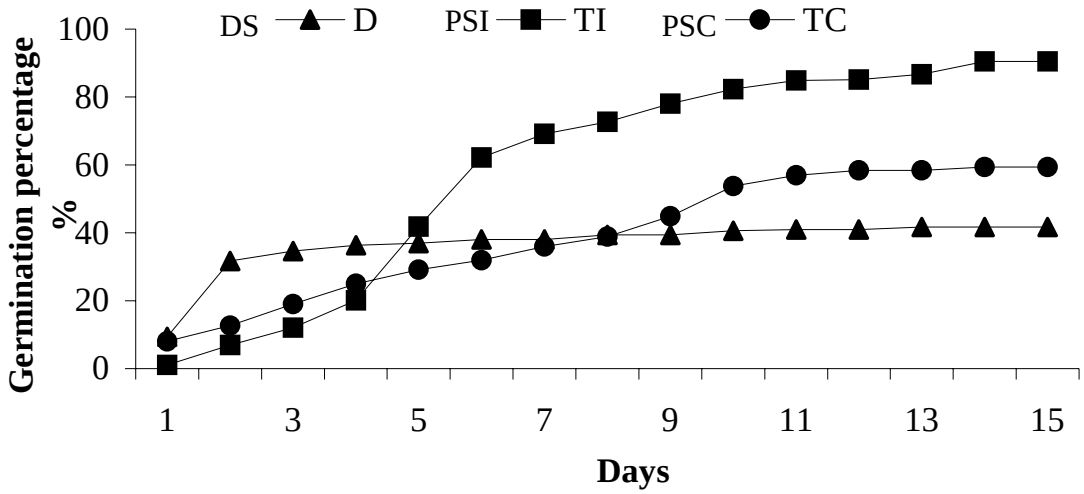

Fig. 3. Variation of the percentage of germination according to the time of diploid seeds (DS) and those polyploid induced by immersion in the solution of colchicine (PSI) of germinated seeds or by meristematic application of cotton soaked by the same solution (PSC), of *T. maritima.*

Polyploidy involved an increase in the size and weight of seeds. Several polyploidy complexes show variation in seed size among ploidy level (Müntzing, 1936; Stebbins, 1971; Halloran & Pennell, 1982; Pundir *et al*., 1983; Evans & Rahman, 1990; Van Dijk & Van Delden, 1990 and Maceira *et al.,* 1993). Phenotypic differences among related plants of different ploidy levels are commonly explained as being the consequences of two basic phenomena: increased DNA content and/or increased heterozygosity (Müntzing, 1936; Stebbins, 1971 and Bennett, 1972). An increase in the quantity of nuclear DNA will have biophysical consequences, independent of the nature of the information carried. This "nucleotypic effect" (Bennett, 1972) is the basis for the strong positive correlation observed between the quantity of nuclear DNA and cell length and volume (Stebbins, 1971; Bennett, 1972; Lewis, 1980 and Tal, 1980). These "gigas effects" can have important effects on many morphological traits, such as pollen diameter, seed size and weight, and the general size of organs such leaves, all of which are greatly enlarged in newly formed polyploids (Müntzing, 1936; Stebbins, 1971 and Bennett, 1972). Nucleotypic effects are also a major source of increased seed weight in polyploids (Müntzing, 1936; Wit, 1959; Stebbins, 1971 and Pundir *et al*., 1983).

A second major change accompanying polyploidization is the so called "genotypic effects" (Bennett, 1972) due to a higher level of individual heterozygosity that can be attained both by an increase in the number of potential heterozygous genotypes for a given locus and by an increase in the number of heterozygous loci (Bingham, 1980). In allopolyploids the combination of homologous genomes may give rise to novel enzyme forms not found in the parental diploids (e.g. in *Tragopogon* (Gottlieb, 1977)). In contrast, in autopolyploids, polysomic inheritance allows for a greater number of alleles and increased heterozygosity per locus in the polyploids (Soltis & Soltis, 1989 and Lumaret & Barrientos, 1990). Such increased heterozygosity may be correlated with increased vigour (Bingham, 1980 and Tomekpe & Lumaret, 1991) and could also enhance seed biomass.

Little is known however of the consequences of increased seed size on germinative capacity in related diploids and tetraploids. The follow-up of germination in our work, showed that polyploidy improved the germination percentage and slowed the germination speed. Indeed, we registered a very significant increase of the germination percentage in polyploid seeds compared with the diploid one, with a more spectacular increase at seeds PSI (90.52% against 41.67%). This increase in the percentage of germination was accompanied by a deceleration of kinetics germinative. A similar result was reported by Bretagnolle *et al.*, (Bretagnolle *et al.*, 1995) which recorded a higher germination percentage of tetraploid plants seeds of *Dactylis glomerata* L. The delay of germination could be allotted to the deceleration of meristematic cells divisions and at a slower speed of cells differentiation (Hirsch, 2001). Several findings underline that early performance can be markedly influenced by seed size variation (Gross, 1984; Stanton, 1984; Wulff, 1986; Roach, 1987). It reported (Bretagnolle *et al.*, 1995) that within a ploidy level, seed weight influenced neither the percentage nor the speed of germination in *Dactylis glomerata*, however there were significant differences between ploidy levels in the speed of germination.

To conclude, although the polyploïdisation caused a deceleration of the germinative phenomenon, we record a clear improvement of the percentage of germination. Hence, polyploidisation may be used as means of improvement of germination. The comparison of the two parameters within the polyploid seeds shows that polyploid seeds induced by immersion (PSI) have a rate and a germination speed higher than the ones induced by the caulinary colchicines application (PSC) and consequently a weaker duration of germination.

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