Studies to Improve Seed Germination in Tetraploids Watermelon (*Citrullus lanatus Thunb*.)

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Abstract- Genetic improvement to produce seedless watermelon offers high quality fruits. This paper describes about problems related with tetraploid seed germination, various seed treatment to improve tetraploid seed germination and the morphological variations observed between the tetraploid and diploid seeds. Diploid watermelon seeds of three genetic backgrounds were treated with colchicine (0.2, 0.3 and 0.4%) and PEG (0.5%) as an adjuvent. Polyploid watermelon seeds have poor germination and low seedling vigor mainly due to thick seed coat and seed coat adherence to emerged cotyledons. In order to enhance the germination, the tetraploid seeds were subjected to five different seed treatments *i.e;* acid scarification, GA_3 treatment, humidification, mechanical scarification and water soaking for 48hrs. Among the various treatments tested, maximum germination was recorded in the seeds subjected to water soaking for 48 hrs in all the three varieties. Tetraploids produce less number of seeds per fruit than those of diploids. Diploid seeds had complete filled cavity with embryo whereas tetraploids developed weak embryo with some empty cavity hence lower seed germination.

Index Terms- Colchicines, Germination, Polyploids, Tetraploid and Water melon

I. INTRODUCTION

Use of triploid hybrids has provided a method for production of seedless fruit . The development of triploid water melon cultivars adds extra time for the development of tetraploid watermelon and additional selection against sterility (Kihara, 1951). Breeders, interested in the production of seedless triploid hybrids, need to develop tetraploid inbred lines and colchicine is probably the most widely used chemical for induction of watermelon tetraploids. Besides low fertility and seed yield, poor seed germination is another problem with tetraploids (Andrus *et al.,* 1971). Hence a small number of tetraploid inbred lines are available. Poor seed germination in polyploid watermelon is generally correlated with thick seed coat, poor embryo and high moisture content (Grange *et al.*, 2000). In many seeds, germination can be inhibited by mechanical restriction exerted by the seed coat. Permeability limitation of water and gases is typical due to hard seed coat. The imbibed coat and large seed cavity in the tetraploid watermelon form a continuous wet layer around the embryo by which the oxygen must transverse (Grange *et al.*, 2003).

Effect of seed treatments on germination of diploid and tetraploid watermelons has been studied (Sung & Chiu, 1995) but little information is available regarding seed treatment effects on tetraploid seed germination, emergence and growth. Because of reduced viability with tetraploid versus diploid watermelon seeds, growers use transplants from seeds that are germinated with some seed treatments in incubators to facilitate uniform germination and improved plant production. Even if polyploid plants are produced with seed enhancements, seedling emergence and growth may still be reduced. It is well known that the establishment of a good seedling stand is a prerequisite for improved yield and quality (Wurr & Fellows, 1983). This research was conducted to determine the effectiveness of seed alteration and chemical treatments on tetraploid watermelon seed germination and morphological variations observed in the polyploid seeds.

II. MATERIAL AND METHODS

The present investigation was carried out during *rabi* and summer seasons of 2012-2013 at the experimental plots of the Division of Vegetable Crops, Indian Institute of Horticultural Research (IIHR), Bangalore, Karnataka, India. Geographically, the experimental site was located at 13°N latitude and 17°37' E longitude at an altitude of 890 m above mean sea level. Soils are red sandy loam with a pH ranging from 5.2 to 6.4. The experiment was laid out in a CRD for each variety with 6 treatments, 3 replications

Seed material: The diploid watermelon seeds of Arka Muthu, SugarBaby and IIHR-14 were treated with colchicine at the cotyledonary stage to induce tetraploidy (Jaskani *et al.*, 2004). Putative tetraploids were identified based on and chloroplast count in the guard cells and observing stomatal density per microscopic area. Thus identified putative tetraploids were self pollinated to obtain M_1 seeds.

1. **Number of seeds per fruit**
   Self pollinated fruits from putative tetraploid plants were harvested at mature stage. The number of seeds present in each fruit were counted and expressed as average number of seeds per fruit for tetraploids in each variety.
2. **Seed length (mm):** Length of ten randomly selected seeds per tetraploid line were recorded using digital vernier calliper and expressed as average seed length.
3. **Seed width (mm):** Width of ten randomly selected seeds per tetraploid line were recorded using digital vernier calliper and expressed as average seed width.

Treatments to improve seed germination of tetraploids:

In order to enhance the germination, the M_1 tetraploid

seeds were subjected to five different seed treatments *i.e.* acid scarification, GA_3 treatment, humidification, mechanical scarification and water soaking. The experiment was laid out in completely randomized design for each variety with 6 treatments, 3 replications and 5 seeds per replication. The number of seeds per replication was limited to 5 as the seed yield per fruit in tetraploids was low and scarce. The treatment details were as follows:

a. Acid Scarification: Tetraploid seeds were soaked in HCl solution (35 per cent) for 3 minutes. Later the seeds were washed thoroughly under running water to remove the residue of the acid from the seed surface. Later those seeds were kept for germination.

b. GA_3 treatment: Tetraploid seeds were subjected to GA_3 treatment by soaking the seeds in GA_3 solution (100ppm) for 48 hrs. Later the seeds were washed thoroughly and kept for germination**.**

c. Mechanical Scarification: Seeds were subjected to mechanical treatment by rubbing the seeds on a rough surface and later those seeds were kept for germination.

d. Humidification: Seeds were subjected to water vapour treatment in an enclosed chamber. Humidification helps in activation of the enzymes and increases the vigour of the seedling.

e. Water Soaking: Seeds were kept for soaking in distilled water for 48 hrs. Later they were washed and kept for germination.

All the treated seeds were placed on germination papers and kept in a germination chamber at 20/30^{o}C (day and night) and 90 per cent humidity. Germination percentage was recorded on 3rd day of sowing.

III. RESULTS AND DISCUSSION

Number of seeds per fruit

Seed yield of tetraploid lines in the early generation was often only 50-100 seeds per fruit compared to 200-800 in diploids (Jaskani *et al*., 2005). Even in the current investigation there was a significant difference between tetraploids and diploids in all the three varieties and the seed yield in tetraploids was less than the diploids (Table 1). Among the tetraploids of three varieties, maximum number of seeds were recorded in the variety Sugar Baby (131.54), followed by Arka Muthu (94.31) and IIHR-14 (51.00). Schwanitz *et al* (1951) suggested that physiological factors were also responsible for the meager fruit set and seed setting in teraploids. He observed that as a result of polyploidy, the number of ovules per ovary was considerably reduced in some plants and this was correlated with low seed set.

Seed length

Significant difference was observed between the tetraploid and diploid seed length in all the varieties studied (Table 2). Tetraploids had a coarse appearance with increased seed length. Among the tetraploids of three varieties, maximum seed length was observed in the variety Arka Muthu (11.91mm), followed by IIHR-14 (9.10mm) and Sugar Baby (7.57mm) and diploid seed length of variety Arka Muthu (6.21mm), IIHR-14 (6.20mm) and Sugar Baby (5.64mm). Hence, it was concluded that there is a consistent significant difference between the diploids and tetraploids with respect to seed length among three varieties. Hence it can be used as a diagnostic trait for tetraploidy. Further, Kihara and Nishiyama (1951) also observed that the seeds of diploids were oblong whereas the tetraploids were varied from oblong to round. Diploid seeds were completely filled but tetraploids showed cavity at the chalazal end of the seed. Similar observations were made in the current study (Plate 1).

Seed width

Significant difference was observed between the tetraploid and diploid seed width in all the varieties studied (Table 3). Tetraploid seeds were wider compared to diploids, and among the tetraploids of three varieties, Arka Muthu seeds had maximum seed width (6.81mm) followed by IIHR-14 (5.61mm) and Sugar Baby (4.58mm). Jaskani *et al.*(2005) also reported that bigger seed size was related to higher ploidy level in watermelon. Hence, it was concluded that there was a consistent significant difference between the diploids and tetraploids with respect to seed width among three varieties. Hence it can be used as a diagnostic trait for tetraploidy.

Treatments to improve seed germination of tetraploids:

In order to enhance the germination, the tetraploid seeds of the three genetic backgrounds were subjected to five different seed treatments *i.e;* acid scarification, GA_3 treatment, humidification, mechanical scarification and water soaking for 48 hrs. Among the various treatments tested, maximum germination was recorded in the seeds subjected to water soaking for 48 hrs in all the three varieties (Table 4). Highest germination was observed in the tetraploids of the variety Arka Muthu (93.30per cent) followed by Sugar Baby (73.30per cent). The tetraploids of IIHR-14 recorded a lower percentage of seed germination (33.30per cent). The percentage of germination was found to be higher in control when compared to the acid scarification and GA_3 treatment. This may be due to the higher concentration of HCl (35per cent) which was used for treating the seeds. Mechanical treatment in the Cv. Arka Muthu and Sugar Baby was found to be on par with the control and the germination percentage was less when compared to the priming treatment. Poor germination of the seeds which are subjected to mechanical scarification may be due to the embryo injury which was caused at the time of the treatment.

IV. CONCLUSION

The investigation conclusively proved that among the various treatments tested, maximum germination was recorded in the seeds subjected to water soaking for 48 hrs in all the three varieties and highest germination was observed in the variety Arka Muthu (93.30per cent) followed by Sugar Baby (73.30per cent) and IIHR-14 (33.30 per cent).

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Table 1: Variation in number of seeds per fruit among tetraploid, mixoploid and diploid lines of watermelon varieties

Ploidy	Arka Muthu	Sugar Baby	IIHR-14
Tetraploid	94.31	131.54	51.00
Mixoploid	65.80	85.3 3	66.00
Diploid	315 .00	261.60	156.62
S.E(m)±	24.90	33.70	18.20
CD (P=0.05)	74.70	101.20	54.70

Table 2: Variation in seed length (mm) among polyploid M_1 lines of watermelon varieties

Ploidy	Arka Muthu	Sugar Baby	IIHR-14
Tetraploids	11.91	7.57	9.10
Mixoploids	11.60	7.12	9.10
Diploids	6.21	5.64	6.20
S.E(m)±	0.5	0.05	0.05
CD (P=0.05)	1.5	0.17	0.15

Table 3: Variation in seed width (mm) among polyploid M_1 lines of watermelon varieties

Ploidy	Arka Muthu	Sugar Baby	IIHR-14
Tetraploids	6.81	4.58	5.61
Mixoploids	6.48	4.56	5.23
Diploids	2.52	2.90	2.21
S.E(m)±	0.33	0.07	0.05
CD (P=0.01)	1.00	0.23	0.16

Table 4 : Germination percentage of M_1 seeds in different seed treatments

Treatment	Arka Muthu	Sugar Baby	IIHR-14
Acid scarification	33.3(30.2)	20.0(17.9)	0(0.67)
GA_3	60.0(51.1)	40.0(34.4)	6.7(9.3)
Humidification	60.0(46.9)	46.6(46.9)	6.6(17.9)
Mechanical scarification	66.6(54.9)	53.3(46.9)	13.3(19.9)
Water soaking for 48hrs	93.3(80.6)	73.3(59.2)	33.3(30.2)
Control	66.6(46.9)	53.3(51.1)	20.0(22.1)
S.E(m)±	9.5	-	-
CD (P=0.05)	28.6 (25.08)	-	-

*Figures in parenthesis indicates Arc sin transformation values

Plate 1 : Empty cavity at the chalazal end of tetraploid seed.