



Genetic Analysis of a Dwarf Vine and Small Fruit Watermelon Mutant

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Received 6 June 2016; Received in revised form 16 August 2016; Accepted 25 October 2016

Available online

Abstract

The dwarf vine and small fruit watermelon, referred to as 'dsh' in this study, is a natural mutant of the ordinary watermelon, 'Hanxuan Lvyuan'. Morphological assessment indicated that its main vine length, internode length, and single-fruit weight were significantly lower than those of the ordinary watermelon ($P < 0.05$). After crossing the mutant with two ordinary watermelons, following by inbreeding the F_1 and backcrossing (BC) F_1 to 'dsh', the F_1 , F_2 , and BC generations were obtained. Genetic analysis of vine type and single-fruit weight showed that the F_1 generation consisted of long-vine plants, with single-fruit weight significantly higher than that of the ordinary parent watermelon ($P < 0.05$). The long and short vine plant ratios of the F_2 and BC populations conform to the Mendel's segregation ratios of 3:1 and 1:1 ($P > 0.05$), respectively. It was suggested that this dwarf and small fruit mutation was a single recessive gene trait that followed a Mendel's inheritance.

Keywords: watermelon; dwarf; small fruit; short vine; mutant

1. Introduction

Watermelon (*Citrullus lanatus*) plays an important role in the melon and fruit crop production (Zhao, 2013). According to the statistics of the Ministry of Agriculture of the People's Republic of China (2013), watermelon production in Henan, Shandong, Anhui, Hunan, Jiangsu, and Hubei Provinces reached 1.8015 million hm^2 in 2012. Internode length is a major plant characteristic of watermelon. Short vine plants with shorter internodes are highly suitable for high-density planting and can save land resources to a certain extent by improving yield per unit area (Ma et al., 2004). However, large-scale conventional cultivated watermelons mainly involve long vine varieties, whereas the short vine watermelons are relatively smaller (Wang, 2014). Developing compact short vine watermelons to selectively propagate high-quality short vine watermelons has become one of the main traits in breeding (Zhang et al., 2010).

Since Mohr (1956) first discovered the short vine watermelon mutant from the long vine watermelon varieties as well as its subsequent relevant genetic studies (Mohr, 1963; Mohr and Sandhu, 1975), other researchers have developed genetic studies of short vine watermelon and cultivation of new varieties (Liu

and Loy, 1972; Bassett, 1986; Dyutin and Afanas'eva, 1987a, 1987b; Sincha, 1998). Although research investigations on short vine watermelon in China began late, recent excavations (Li et al., 1993; Yang and Li, 2009), mutagenesis (Zhang et al., 2010), genetic analysis (Huang et al., 1998; Ma et al., 2004), and evaluation of prospective applications (Huang et al., 1995; Yang, 2010) of the short vine watermelon mutant have made significant progress. According to a review conducted by Yang (2010), 4 known loci, *dw-1*, *dw-2*, *dw-3*, and *dw-4*, regulate short-vine watermelon trait, most of which are single-gene recessive mutations. In addition, Ma et al. (2004) conducted the genetic analysis of two short-vine mutants that were regulated by two recessive genes, namely, *dw-1/dw-1* and *dw-2/dw-2*. However, the short-vine mutants still have some undesirable traits, and its genetic improvement and the development of applications have not been extensively and systematically studied (Zhang et al., 2010).

In 2009, we identified the watermelon, 'Hanxuan Lvyuan' (Code I911, inbred hybrid selected 7th generation), which developed shorter vines, produced small leaves and fruits, as well as growing under normal conditions, hereby named 'dsh'. In the present study, we conducted crossing, self-crossing, and back-cross experiments of the mutant 'dsh' with two normal long vine

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Peer review under responsibility of Chinese Society for Horticultural Science (CSHS) and Institute of Vegetables and Flowers (IVF), Chinese Academy of Agricultural Sciences (CAAS)

The Chinese version of this paper is published in Acta Horticulturae Sinica. doi:10.16420/j.issn.0513-353x.2015-0754

<http://dx.doi.org/10.1016/j.hpj.2016.10.002>

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watermelons to establish the F_1 , F_2 , and BC populations. By analyzing the traits of different populations, this study revealed the classical trait and inheritance model of the short-vine mutant 'dsh' for the effective application of genetically improved short-vine mutants.

2. Materials and methods

2.1. Experimental materials

'Hanxuan Lvyan' (Code I911), its small vine and dwarf mutant 'dsh', and one normal long vine (Code I912) watermelon were cultivated and stored at the Laboratory of Plant Germplasm and Genetics, College of Life Sciences, Henan University, China.

All field tests were conducted at the Genetics and Breeding Bases of Henan University. Matrix potting and a growth in small plastic arch shed nursery were performed from 2009 to the end of March 2014. In late April 2014, the seedlings at the third-leaf stage were grown in the tested fields with 2 m row spacing and 0.4 m plant spacing and mulching in accordance with conventional field management schemes.

2.2. Obtaining different generations

After the discovery of the dwarf mutant 'dsh' in 2009, these were self-crossed to maintain purity (P_2) or pollinated on the female flower of I911 (P_1), followed by crossbreeding after fruit ripening to obtain hybrid seeds (F_1). The F_1 was planted in 2010 and self-crossed to obtain the F_2 population, and 'dsh' was used as the paternal plant in a backcross to obtain BC population. In 2011, F_2 and BC seeds were planted to observe segregation at the vine growth stage. To further reveal this mutation trait and inheritance, we crossed and backcrossed the mutant 'dsh' (P_2) with I911 (P_1) and I912 (P_3) and collected the seeds of the F_1 population ($P_2 \times P_1$, $P_1 \times P_2$, $P_2 \times P_3$, and $P_3 \times P_2$) after fruit ripening. We observed and compared the morphological indicators of P_1 , P_2 , and F_1 in 2013. In addition, the F_1 population was self-crossed at flowering stage to yield F_2 seeds, and 'dsh' was used as the paternal plant in a backcross to obtain BC seeds. In 2014, we planted P_1 , P_2 , P_3 , F_1 ($P_1 \times P_2$), F_1 ($P_2 \times P_3$), F_2 [$(P_2 \times P_1) \otimes$, ($P_1 \times P_2$) \otimes , ($P_2 \times P_3$) \otimes , ($P_3 \times P_2$) \otimes], and BC [$(P_2 \times P_1) \times P_2$, ($P_1 \times P_2$) $\times P_2$, ($P_2 \times P_3$) $\times P_2$, ($P_3 \times P_2$) $\times P_2$] populations to analyze the segregation of dwarf vine and small fruit traits.

2.3. Determination of morphological indicators and fruit weight and statistical analysis

Nine healthy plants of P_1 , P_2 , and F_1 ($P_2 \times P_1$) plants were selected in April 2013, and were subsequently used in the measurement of the length, width, petiole length, main vine length, diameter, number of inter-nodes, and inter-node length of the fifth main vine leaves at 30 d and 60 d after transplanting and to determine the single-fruit weight after fruit ripening. In April 2014, 3 to 6 healthy plants from each of the P_1 , P_2 , P_3 , F_1 ($P_1 \times P_2$), and F_1 ($P_2 \times P_3$) populations, and 20 to 30 long vine plants and 10 to 15 short vine plants of F_2 population were selected and used in the determination of single-fruit weight after fruit ripening, while the hybrid combinations of $P_2 \times P_1$ and $P_3 \times P_2$ were only observed and recorded their vine types.

The SPSS 19.0 (SPSS, Inc., Chicago, IL) software was used for statistical analysis. The measurement results were analyzed using a One-way ANOVA and multiple comparisons were performed using the Duncan's method.

2.4. Genetic analysis of the dwarf and small fruit mutants

The three-leaf stage seedlings of F_2 , BC, P_1 , P_2 , and P_3 were planted in late April 2014. A randomized block design was used and repeated three times. Two plants were planted per hole with 1 m row spacing, 0.2 m plant spacing, and mulching. Morphological statistics were conducted in each generation of vine plants during the flowering and fruit setting stages, followed by the Chi-Square test. In order to obtain normal fruit, some of the plants were removed to make the rest plants were planted with 2 m row spacing and 0.4 m plant spacing.

3. Results

3.1. Comparative analysis of morphological indicators of ordinary watermelon (I911) and its dwarf vine and small fruit mutant ('dsh')

Compared to the morphological traits of the watermelon 'I911', the dwarf vine and small fruit mutant 'dsh' showed short vines, thin stems, and numerous branches (Fig. 1). In addition, the primary leaf blade was normal with a small leaf size beyond the leaf center and a warped leaf margin. The organs of the female flowers showed normal development but were smaller in size, which was approximately one-third of that of 'I911'. The number of male and female flowers was relatively high. The fruit shape, skin color, and striped appearance of the 'dsh' were similar to those of its parents; however, the volume of its fruit was relatively smaller.

To further reveal the morphological differences between 'I911' (P_1), 'dsh' (P_2), and its F_1 hybrids, we comparatively analyzed the length, width, petiole length of the fifth main vine leaves, and fruit weight at the mature stage at approximately 30 d and 60 d after planting (Table 1). The results showed that the length, width, petiole length of the fifth main vine leaves, and the single-fruit weight of 'dsh' (P_2) were significantly different from those of the 'I911' (P_1) and its F_1 hybrids ($P < 0.05$). The length, width, petiole length of the fifth main vine leaves of the 'dsh' (P_2) were respectively only 66.1%, 67.5%, 42.0% and 54.9%, 58.0%, 43.6% of those of the I911 (P_1) at 30 d and 60 d after planting. The single-fruit weight of the 'dsh' (P_2) was 19% of that of the I911 (P_1). Despite the significant differences in blade length and inter-node number between F_1 and I911 (P_1) generations, the blade width, petiole length, main vine length, inter-node length, and fruit weight of the F_1 generation did not significantly differ from those of the I911 (P_1) generation, thereby indicating that the traits of the mutant 'dsh' fruits were regulated by recessive genes.

3.2. Genetic analysis of genes associated with dwarf vine and small fruits in the 'dsh' mutants

The vine type of F_1 , F_2 , and BC populations of 'dsh' (P_2) and two normal long vine watermelon 'I911' (P_1) and 'I912' (P_3) were observed and recorded, then a Chi-Square Test was conducted. The results showed that F_1 generation all developed long vines,

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