Vikrant Tyagi* and S. K. Dhillon Effect of Alien Cytoplasm on Combining Ability for Earliness and Seed Yield in Sunflower under Irrigation and Drought Stress

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Abstract: Nine CMS analogues from different cytoplasmic sources and their common maintainer were crossed with four perfect restorers from PET-1 source to develop of 40 sunflower hybrids. The experiment was conducted during spring season 2011 and 2012 two years continuously, under two different water environments, normal irrigated and water stress environment, where irrigation was stopped after the anthesis. The analysis of variance revealed significant differences among the sources for day to flowering, days to maturity and seed yield under both the environments, both the years and pooled over the years. The variance due to GCA and SCA showed that gene action was additive for maturity whereas, non-additive genetic effect was observed for days to flowering and seed yield under both the environments. The CMS analogues E002-91A (H. annuus), PKU-2A (H. annuus), and ARG-3A (H. argophyllus) were recorded as good combiner for early flowering in both the environments. The CMS analogs E002-91A and ARG-3A were observed good combiners for early flowering, early maturity and high seed yield, whereas, NC-41B from conventional CMS source H. petiolaris (PET-1) was recorded good combiner for late flower and early maturity and poor combiner for seed yield under both the environments. Among testers P69R was recorded good combiner for late flowering under normal water environment while, in water stress it was good combiner for early flowering but it was not good for seed yield. RCR-8297 was recorded for good combiner for late maturity and high seed yield under both the environments. The SCA estimates were highest in cross E002-91A × RCR-8297 and PKU-2A × P124R and ARG-6A × RCR-8297 for early flowering and early maturity under both the environments. These new cytoplasmic male sterility sources can be substitute of the classical (PET-1) source with added advantage for early maturing and high yielding.

^{*}Corresponding author: Vikrant Tyagi, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab 141004, India, E-mail: vikranttyagi97@gmail.com S. K. Dhillon, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, Punjab 141004, India

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Introduction

The discovery of cytoplasmic male sterility (CMS) in sunflower by Leclercq (1969) and subsequent identification of genes for fertility restoration have resulted in the development of commercial hybrids since 1972. However, all the sunflower hybrids that are commercially grown have a single source of CMS discovered by Leclereq leading to homogeneity and potential risk that was evident in case of maize. CMS-T was exclusively used for maize hybrid-seed production in the United States and it became susceptible to Southern corn leaf blight (SCLB), caused by race T of the fungus (Ullstrup, 1972). As a result, in 1970, maize crop suffered colossal yield losses caused by the SCLB epidemic (Ullstrup, 1972). Diversification of CMS source is inevitable in heterosis breeding programs as the use of a single CMS source involves a potential risk if it became susceptible to a new strain of disease. In order to diversify the cytoplasmic base, attempts have been made and several new cytoplasmic sources have been identified. The present investigation is based on development of a set of nine CMS analogues having a common maintainer and identification of four common restorers from PET-1 source for all these sources. Only a few studies have reported the interactions between cytoplasms and nuclear genes in the expression of several qualitative and quantitative traits in sunflower. In sunflower a unique cytoplasmic nuclear interaction had caused reduction in chlorophyll, photosynthetic rate (Jan, 1990) and positive effects on oil content have been reported (Serieys, 1992; Tyagi and Dhillon, 2014). Sunflower (Helianthus annuus L.) as one of the most important sources of vegetable oil in the world, it is moderately tolerant to water stress and its production is affected by drought conditions (Pasda and Diepenbrock, 1990). Although sunflower is moderately tolerant to water stress, its production is greatly affected by drought. Evidence indicates that drought stress during the vegetative phase, flowering and seed-filling period in sunflower causes a considerable decrease in yield and oil content (Ali et al., 2009). Different CMS sources in sunflower were developed at Punjab Agricultural University, Ludhiana and evaluated for their performance for different agronomic, physiological, yield and quality traits under normal irrigated environment (Tyagi et al., 2013, 2015a) as well as under water stress conditions (Tyagi et al., 2015b; Tyagi and Dhillon, 2016a). Sunflower hybrids developed and evaluated from these CMS sources for combining ability for agronomic and yield traits under different irrigation environments (Dhillon and Tyagi, 2016; Tyagi and Dhillon, 2016b). The present study was therefore aimed to study the effect of cytoplasmic male sterility sources on combining ability for earliness and seed yield under normal irrigated and water stress environments to exploit them in hybrid development program for early maturing high yielding sunflower hybrids.

Materials and methods

The present investigation was carried out in the research fields of the oilseeds section, Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana, India. A set of nine CMS analogues from different cytoplasmic sources were developed using a common maintainer (for details, see Table 1) and the detailed procedure for the development of all these CMS analogs has been reported by Tyagi and Dhillon (2016a). Four common perfect restorers from PET-1 source for these CMS sources were identified. All nine CMS analogues along with NC-41B (common maintainer) were crossed with four common restorer lines (to develop the set of 40 hybrids) to study the effect of different cytoplasmic sources on performance of hybrids. Also the efforts were made to identify some good combining CMS analogues for early maturity and high seed yield.

Table 1: Information of different CMS sources along with common maintainer line (NC-41B) developed at PAU, Ludhiana.

The experiment was conducted during spring season in the year 2011 and 2012. The material was raised in paired rows of 4.5 m length with 0.60 m and 0.30 m inter and intra row spacing respectively, in the randomized block design, with three replication for each treatment. All the agronomic practices recommended for the region were followed to raise a good crop. The data for early maturity like day to flowering and days to maturity were recorded on the basis of total plants per genotype whereas seed yield per plant were recorded for five random plants in the field.

The data recorded was statistically analyzed following standard procedures for the estimation of components of genetic variation for each irrigation level separately and for pooled over the different environments. Combining ability analysis was done in Line × Tester analysis, as suggested by Kempthorne (1957).)

Results and discussion

The combining ability analysis, (pooled over the years) presented in Table 2 reveals that the mean squares due to years were highly significant for days to flowering and seed yield under both the environments, while, mean squares for days to maturity under normal environment were non significant. Mean squares due to restorers were non-significant for days to flowering under stress

Table 2: Analysis of variance for combining ability under normal irrigation and stress environment (individual and pooled over years).

*, **Significant at 0.05 and 0.01 of probability, N: Normal environment S: Stress environment.

environment. The differences among the female, female \times male, female \times years and male \times years were observed to be highly significant for days to flowering, days to maturity and seed yield, while mean square due to female \times male \times years was non-significant for days to flowering and maturity under normal environment indicating the existence of wider genetic differences among parents. The main role in the inheritance was played by the non-additive component of genetic variance as shown by analysis of variance of combining ability and analysis of genetic variance components. GCA/SCA ratio for days to flowering and seed yield (g/plant) recorded in F_1 generation was below the value of unity under both normal and stress environment. While, days to maturity recorded in F_1 generation was above the value unity in normal and stress environment showed additive component of genetic variance (Table 2). Combining abilities and gene action for different agronomic traits have been estimated by many researchers (Hladni et al., 2006; Shankar et al., 2007; Chandra et al., 2011; Memon et al., 2015; Dhillon and Tyagi, 2016; Tyagi and Dhillon, 2016b). Additive gene action for these traits has also been reported by Singh et al. (1989).

Effect of different cytoplasmic sources on combining ability under different environments (pooled over years)

The importance of combining ability in selection of parents for hybridization has been emphasized by many workers in sunflower (Putt, 1966; Giriraj et al., 1987). The potentiality of any line to be used as a parent in hybridization depends on it is *per se* performance and the performance of F_1 hybrid derived from it and its own GCA effect. The estimates of the general combining ability (GCA) and specific combining ability (SCA) effects for the phenological and yield traits (pooled over the years) under two different water regimes is presented in Table 3, 4 and 5.

Days to flowering

In normal irrigated environment, the CMS analogues E002-91A (H. annuus), PKU-2A (H. annuus), ARG-2A (H. argophyllus), ARG-3A (H. argophyllus), DV-10A (H. debilis ssp vestitus) and PRUN-29A had highly significant negative GCA effects $(-0.57, -0.45, -1.45, -0.95, -1.37, -0.70)$ respectively and were observed as very good combiners for early flowering. Two of the CMS analogues

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Table 5: Estimation of combining ability effects and mean performance of hybrids for seed yield under normal and water stress environment. (Pooled

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CMS-XA and ARG-6A having GCA effects of -0.37 and -0.32 (significant at 5 per cent) were identified as good combiner for early flowering. Whereas CMS PHIR-27A (H. praecox ssp hirtus) had highly significant positive GCA effects (1.70) and hence was recorded as very good combiner for late flowering. The maintainer line NC-41B (1.22) from PET-1 source was very good combiner for late flowering.

In stress environment the CMS analogues E002-91A, PKU-2A and ARG-6A $(GCA \text{ effects of } -1.65, -2.19 \text{ and } -0.85)$ were recorded as very good combiner while ARG-3A (*H. argophyllus*) and PHIR-27A (*H. praecox ssp hirtus*) (-0.57 and $-$ 0.65) were good combiner for early flowering. The CMS analogue ARG-6A (0.85) and NC-41B (3.56) from PET-1 source had significant positive GCA effects under stress environment and were recorded as very good combiner for late flowering. The CMS analogues E002-91A, PKU-2A and ARG-3A (H. argophyllus) were recorded as good combiner for early flowering in both the environments. Among restorers the male parent P124R was recorded as very good combiner for early flowering under normal environment, whereas P69R was very good combiner for late flowering in normal environment while very good combiner under water stress environment for early flowering.

The cross combinations viz. $E002-91A \times RCR-8297$ (-1.49 and -1.24), PKU-2A × P124R (-0.85 and -1.92) and ARG-6A × RCR-8297 (-1.99 and -1.07) having highly significant SCA effects were identified for early flowering under both the environments. The cross PKU-2A \times P100R (0.88 and 1.22) recorded highly positive SCA effects under both the environments for late flowering.

Days to maturity

The CMS analogues CMS-XA, E002-91A, ARG-3A (H. argophyllus) and PRUN-29A were observed as very good combiner for early maturity due to their highly significant negative GCA effects $(-2.35, -1.12, -0.60, -0.78)$ respectively whereas PKU-2A and DV-10A (H. debilis ssp vestitus) was recorded as good combiner due to their significant GCA effects $(-0.43 \text{ and } -0.35)$ for earliness under normal environment. Only the CMS line PHIR-27 was recorded as very good combiner (1.49 and 1.34) for late maturity under both environments respectively. Under stress environment highly significant negative GCA effects $(-0.54, -0.87$ and -2.33) were recorded for CMS-XA, DV10A, PRUN-29A respectively and were recorded very good combiners for early maturity in stress environment, whereas, ARG-3A and ARG-6A from $(H. \, \text{argophyllus})$ were recorded as a good combiner (–0.46 and 0.42) for earliness and late maturity respectively. The CMS lines from PET-1 source NC-41B (-2.62) was observed very good combiner for early maturity. The male parent RCR-8297 with GCA value of 0.42 and

0.88 was recorded as a very good combiner for late maturity, while P124R with GCA value of $(-0.43 \text{ and } -0.45)$ was observed as very good combiner for earliness under both the environments respectively. The male parent P69R average combiner under normal environment due to its non-significant negative GCA effect (-0.14) under normal environment whereas, very good combiner (-0.40) for earliness under stress environment. Significantly negative GCA effects for maturity were also reported by Kang et al. (2013) and Saleem et al. (2014). These CMS lines and male lines may also be used in sunflower heterosis breeding to get early matured hybrids.

The cross combinations $E002-91A \times RCR-8297$ (-1.61 and -4.17), PKU- $2A \times P124R$ (-2.53 and -1.30), DV10A \times P69R (-1.48 and -2.14), DV-10A \times P100R $(-1.28$ and $-1.18)$ and PRUN-29A × P100R $(-1.51$ and $-1.22)$ were recorded as having high SCA effects for earliness under both the environments. While cross combinations $E002-91A \times P124R$ (1.08 and 1.32), $E002-91A \times P100R$ (1.16 and 2.07), PKU-2A × RCR-8297 (0.78 and 3.04) and DV-10A × RCR-8297 (1.70 and 2.75) were recorded high SCA effect for late maturity under both the environments. The earlier findings of Limbore et al. (1997) and Bajaj et al. (1997) were in conformity with the present findings.

Seed yield

The CMS analogues E002-91A (1.75), ARG-3A (5.17) and ARG-6A (7.21) from (H. argophyllus) having highly significant positive GCA effects were recorded as very good combiner, whereas, NC-41B from $PET-1$ (-5.24) with negative GCA effects was poor combiner for seed yield under normal environment. Under stress environment the CMS analogues CMS-XA (6.60), E002-91A (1.82), ARG-3A (H. argophyllus) (4.80), ARG-6A (H. argophyllus) (3.78) and PRUN-29A (8.98) were recorded as very good combiner because these had highly significant GCA effects for seed yield. The CMS analogues E002-91A (H. annuus), ARG-3A (H. argophyllus) and ARG-6A (H. argophyllus) recorded very good combining ability under both the environments. The testers RCR-8297 was recorded very good combiner (3.15 and 1.70) under both the environments, while P100R appeared to be very good general combiner (2.44) for seed yield per plant under normal environment only. While, the maintainer line NC-41B (-5.24 and -15.49) and CMS line ARG-2A (-2.9 and -3.82) performed negative general combining ability effects for seed yield under both the environments respectively. Among the testers P69R (–3.5) and P124R (–2.1) showed highly significant negative GCA effects under normal environment whereas, significant negative GCA under stress environment. Results indicate these newly identified divers CMS sources may be use in future sunflower hybrid breeding programs due to its

high positive GCA performance for seed yield under both the environments. For seed yield good general combing lines have been reported by Kandhola *et al.* (1995), Reddy and Madhavi (2005), Hladni et al. (2006), Parameshwarappa et al. (2008).

Hybrid combinations CMS -XA \times P100R (6.47 and 5.93), ARG-2A \times P100R (9.97 and 10.70), ARG-6A × P69R (5.46 and 4.99), DV-10A × P100R (3.20 and 7.90) and PRUN-29A × RCR-8297 (6.70 and 2.83) were identified with high SCA effects for seed yield per plant under both the environments. Khan et al. (2009), Karasu et al. (2010) were reported significant positive SCA effects for oil content, seed yield and yield associated traits.

The CMS analogs E002-91A (H. annuus) and ARG-3A (H. argophyllus) were observed good combiners for early flowering, early maturity and high seed yield derived from different wild CMS sources performed good for these traits as compared to NC-41B from classical (H. petiolaris) PET-1 source under both the environments, these new sources can be utilize for development of high yielding and early maturing sunflower hybrids with stable performance under normal and water stress environment. These new cytoplasmic male sterility sources derived from different wild spp. can be substitute of the classical (PET-1) source with added advantage for early maturing and high yielding.

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