Annals of Agricultural Science xxx (2017) xxx–xxx

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Contents lists available at ScienceDirect

Annals of Agricultural Science

journal homepage:

Induction of apomixis and fixation of heterosis in Egyptian rice Hybrid1 line using colchicine mutagenesis

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ARTICLE INFO

Article history:
Received 15 December 2016
Received in revised form 7 March 2017
Accepted 22 March 2017
Available online xxxx

Keywords:
Apomixis
Heterosis
Flow cytometry
Colchicine
Rice
Hybrids

ABSTRACT

It is known that hybrid rice yields 15–20% over inbred varieties in first generation because of heterosis. However, heterosis is normally broken due to segregation. Applying apomixis produces plants as a clone of mother plant and overcomes the problem of breaking heterosis. In order to fix heterosis in the Egyptian rice Hybrid1, their seeds were mutagenized in 0.2% colchicine for two time periods 24 and 50 h. After colchicine mutagenesis, rice seedlings were grown in the field till maturation and the resulted M1 seeds were sown in season 2 and plants were selected based on yield and homogeneity. Then, seeds were sown to be evaluated in season 3. Pollen fertility test, esterase isozyme analysis, and flow cytometry seed screening were performed to confirm the results of field selection of populations identical to control. Pollen fertility examination was performed on the populations of the third season. Pollens of populations 304, 298, 292, 284, 281, 154 and 149 were found to be completely sterile. However, these plants had high seed set percentage. The flow cytometry screening of the six yield-based identical populations and the control seeds showed that populations 220, 339, 351 and 298 have higher nuclear DNA content (C2) than untreated hybrid (C2 & C3). Results of flow cytometry clearly showed that population 298 has one peak (C2) and its endosperm was formed autonomously without fertilization. Although its pollen grains were sterile, it showed high seed set percentage. This indicates that heterosis was completely fixed by apomixis in this population.

Introduction

Apomixis is a kind of somatic manipulation (Bicknell and Catanach, 2015), which permits plant breeders to exploit heterosis. Apomixis maintains all the mother genetic makeup and produces an identical clone to mother. Depending on the origin of embryos, it was categorized into gametophytic apomixis and adventitious embryony. In the adventitious embryony, mature seeds usually contain both sexual and asexual embryos as in mango. It is formed from both somatic cells within the ovary (Wakana and Uemoto, 1988) and megaspore mother cell. In the gametophytic apomixis, mature seeds contain only one asexual embryo. This embryo is developed whether from megaspore mother cell, which failed to enter meiosis or enter meiosis but cytokinesis was inhibited (diplospory), or from a somatic cell in nucellar tissue (apospory) (Crane, 2001).

To breed for diplospory, chemicals that cause chromosome doubling or mutagenesis have been applied. They prevent megaspore mother cell (MMC) from entering meiosis and bypass mitosis directly or impedes cytokinesis after entrance megaspore mother cell to meiosis (Quarin et al., 2001), which produced individuals identical to mother plant. Many chemicals are shown to induce chromosome doubling, which resulted in polyploidy in plants, e.g. EMS (ethyle methyle sulfonate), colchicine, triflouraline, NO gas, gamma radiation, electric and magnetic fields. Generally, colchicine, triflouraline and EMS provide variability and improve plants without disrupting characters (Roychowdhury and Tah, 2011; Kannan et al., 2015). For example, in *Citrus*, colchicine was selected for generating polyploidy (Aleza et al., 2009).

Among these chemicals, colchicine was the most successfully and widely used in many species as cotton, maize and wheat as

Peer review under responsibility of Faculty of Agriculture, Ain-Shams University.
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http://dx.doi.org/10.1016/j.aoas.2017.03.001
0570-1783/

well (Mathusamy and Jayabalan, 2002). The success rate in using this chemical for chromosome doubling varied depending on species, concentration and treatment duration (Petersen et al., 2002). Colchicine was applied in different doses and periods on various species and tissues. 0.06–0.5% of colchicine has been applied to maize seedlings (Han et al., 2006). *Sorghum bicolor* was treated with 0.5, 0.2, and 0.1% colchicine (Ghaffari, 2006), while sweet sorghum was treated with different concentrations of colchicine (0–0.1%) for 6, 48 and 72 h (Delnavaz et al., 2010). Recently, Gautam and Kumar (2013) utilized 0.2, 0.4 and 0.6% colchicine treatments to induce cytomixis in *Brassica* spp. Different colchicine concentrations (0.25%, 0.50%, 0.75%, 1.0%, 1.50%, and 2.5%) were used to induce polyploidy in stevia (Rameshsing et al., 2015). Sourour et al. (2014) have used colchicine to produce tetraploid in barley (*Hordeum vulgare* L.). In addition, colchicine was used for doubling the chromosome number of *Calendula officinalis* L. (El-Nashar and Ammar, 2015). Nura et al. (2013) studied the morphological characterization of colchicine-induced mutants of sesame (*Sesamum indicum* L.). Moreover, Murali et al. (2013) have used two different colchicine concentrations (0.1% and 0.2%) to treat sorghum seeds for 24, 48 and 72 h.

It is well known that colchicine is an alkaloid, which during cell division binds to tubulin protein of the spindle fiber and stops microtubules formation (Molad, 2002). Additionally, during meiosis it prevents chromatids separation (Tambong et al., 1998) and inhibits cytokinesis (Antoccia et al., 1993). Moreover, colchicine leads to meiosis aberrations (Souza et al., 2015), which produces aberrant microspores, pollen sterility (Soodan and Wafai, 1987), ovule sterility (Gautam and Kumar, 2013), as well as loss of fertility (Rai et al., 2010).

Polyploid plants can be identified through morphological, cytological, biochemical, and molecular parameters (Omezzine et al., 2012). There are many methods used to identify apomixis, none of them separately has provided a complete picture. Among these methods, biochemical methods (Khokhlov, 1970), progeny test, cytological, histological methods (Bashaw and Hanna, 1990) and flow cytometry (Matzk et al., 2000) were used to recognize apomixis. Therefore, a combination of these methods was applied to accurately categorize the progeny.

Usually, progeny test is used to screen for segregation in mutant populations and it is carried out on seedlings or fully-grown plants. Progeny tests are based on screening of morphological characters using plant height, days to 50% flowering, yield, and yield components (Hanna et al., 1973).

The current study was conducted in order to induce apomixis and fix heterosis in the sterile Egyptian Hybrid1 line using 0.2% colchicine as well as assessment of agronomic & yield characters, and pollen fertility of the identical-mutant populations compared with their parents.

Material and methods

This study was carried out at the experimental farm of Rice Research and Training Center (RRTC), Sakha, Kafr El-Seikh, Egypt, in seasons 2011, 2012 and 2013. The biochemical assay was performed at Genetics laboratory, Botany department, Faculty of Science, Tanta University, Egypt. The flow cytometry assay was conducted at Anatomy department, Faculty of Veterinary, Kafr El-Seikh University, Egypt.

Material

Seeds of *Oryza sativa* L. genotype were obtained from Rice Research and Training Center (RRTC), Sakha, Kafr El-Seikh, Egypt. In this study, the rice genotype Egyptian Hybrid1 was used. Egyptian Hybrid1 is the first three-line hybrid system in Egypt. It is a hybrid of IR 69625 A/B (female parent) and Giza178 (male parent).

Methods

Colchicine mutagenesis

In growing season 2011, 80 seeds of the Egyptian Hybrid1 were mutated. Of which forty seeds were soaked in 0.2% colchicine for 24 h and another forty seeds were treated for 50 h. After colchicine treatment, 23 seedlings only survived. They were washed well with tap water, and then cultivated in a tray containing a clay soil in the RRTC green house. Afterwards, the 30 day old seedlings were transplanted as single plants in the field. The rows were transplanted with one seedling per hill and the spaces of 20 cm between rows and 20 cm between plants were used. Seedlings were transplanted in rows as follow: Egyptian Hybrid1 seedlings (from seeds mutated for 24 h) were grown in two rows (16 plants) and Egyptian Hybrid1 seedlings (from seeds mutated for 50 h) were grown in one row (6 plants). The length of each row was 4 m. All agricultural practices were made according to the recommendation of RRTC: i.e. normal fertilizer level (95.24 kg N and 35.71 kg P_2O_5/ha) and insecticide treatment was applied using carbofuran (Furadan, 15 kg/ha). In addition, weeds were chemically controlled by adding a dose of thiobencarb (Saturn) at the rate of 7.5 L/ha four days after transplanting and no chemicals were used after that.

Progeny test

Yield components were recorded for the 23 survived individuals, and their seeds were kept to be cultivated in the following seasons. In season 2012, seeds of 23 lines (entries) were planted each in a row. Thirty-day old seedlings were moved to be transplanted in separate plots. Each plot was three rows of 4 m length, with spacing of 20×20 cm between rows and hills. All agricultural practices were applied according to RRTC recommendations.

247 mutant plants, showing high yield and earliness compared to Egyptian Hybrid1 (i.e. same or better than their parent vigor), were selected and grown in the following season to evaluate their similarity. All agronomic and yield characters (days to flowering, total weight per plant, yield per plant, yield per 1 m^2, 1000-grains weight, seed set percentage, panicle length, and plant height) were documented.

In 2013 season, the selected plants (247 plants) and their untreated mother parent plants were transplanted in a completely randomized design. Each plant line was considered as a population. The seeds of each selected plant line were sown in three rows. All transplanting rules and agricultural practices were applied following RRTC recommendations. All the selected agronomic and yield characters were recorded.

Pollen fertility examination

At the flowering stage of 2012 and 2013 seasons, flowers were picked up and fixed in the fixative Carnoy's solution (3 ethanol: 1 acetic acid) until microscopic fertility examination was done. Anthers were dissected and gently squashed. Then, in order to examine pollen fertility, iodine was used as staining dye of the pollen grains.

Flow cytometry screening of rice seeds

Mature dried seed samples, which were collected at the third season, from the mutant plants (produced by 0.2% colchicine treatment of Egyptian Hybrid1 genotype) were chopped with a razor blade in a Petri-dish containing cold ice, 2 µl of DAPI staining buffer (DNA staining solution from KPL, USA) and 998 µl of nuclei lysis buffer (0.5 M Tris, 2.5 mM $MgCl_2$, 85 mM NaCl, and 0.1% (v/v) Tri-

Please cite this article in press as: Gaafar, R.M., et al. Induction of apomixis and fixation of heterosis in Egyptian rice Hybrid1 line using colchicine mutagenesis. Ann. Agric. Sci. (2017), http://dx.doi.org/10.1016/j.aoas.2017.03.001