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Diversification of primary gene pool through introgression of resistance to foliar diseases from synthetic amphidiploids to cultivated groundnut (*Arachis hypogaea* L.)

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ABSTRACT

Groundnut (*Arachis hypogaea* L.) is widely grown and consumed around the world and is considered to have originated from a single hybridization event between two wild diploids. The utilization of wild germplasm in breeding programs has been restricted by reproductive barriers between wild and cultivated species and technical difficulties in making large numbers of crosses. Efforts to overcome these hurdles have resulted in the development of synthetic amphidiploids, namely ISATGR 278-18 (*Arachis duranensis* × *Arachis batizocoi*) and ISATGR 5B (*Arachis magna* × *A. batizocoi*), which possess several desirable traits, including resistance to foliar diseases that generally cause huge yield losses annually in groundnut growing areas of Asia, America, and Africa. With an objective to improve foliar disease resistance, the primary gene pool was diversified by introgressing foliar disease resistance in five cultivated genotypes (ICGV 91114, ICGS 76, ICGV 91278, JL 24, and DH 86) from synthetic amphidiploids using a backcross breeding approach. Several introgression lines with resistance to two foliar diseases (rust and late leaf spot) were identified with levels of resistance equal to the donors. These backcross derived lines have shown a wide range of variation for several morphological and agronomic traits. These lines, after further evaluation and selection, can serve as donors in future breeding programs aimed at

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developing improved cultivars with desirable agronomic traits, high resilience to biotic/abiotic stresses and a broadened genetic base.

1. Introduction

Cultivated groundnut (*Arachis hypogaea* L.), also known as peanut, is grown on nearly 24 million hectares of land area globally with an annual production of 38 million tons (Mt) [1]. Although it originated in South America, the vast majority of groundnut is produced in Asia (68%, 23 Mt) and Africa (24%, 8 Mt), whereas the remaining (8%, 3.5 Mt) comes from North America, Caribbean countries, Europe and Oceania [1]. Besides being a major source of vegetable oil and providing several confectionary preparations, this crop is also a principal source of nutrition by providing human dietary protein, oil/fat, and vitamins such as thiamine, riboflavin and niacin in parts of Asia and Africa [2]. Additionally, it provides an important livestock feed along with improving soil fertility through contributing up to 60 kg ha^{-1} of nitrogen to the soil [3].

Surmounting biotic and abiotic pressure along with the narrow genetic base of the cultivated gene pool has seriously reduced the crop potential and hampered the possibility of meeting future demands of continuously increasing human and animal populations [4,5]. Control of drought stress and foliar diseases requires urgent attention in order to sustain productivity in the fields of resource-poor farmers. Foliar diseases such as late leaf spot (LLS) caused by *Cercosporidium personatum* and leaf rust caused by *Puccinia arachidis* are important diseases of groundnut in Africa, Asia, and the Americas [6,7]. The extent of economic loss due to LLS [8] may be much higher than the reported global yield loss of 600 million US$. Disease management through application of fungicides is not a viable option for resource-poor farmers; also, fungicides may pollute the environment and ground water besides causing greater risk and damage to crop [7]. Hence, the only eco-friendly approach is to equip popular cultivars with resistance genes that will ensure sustainable resistance against foliar fungal pathogens.

Molecular analysis has shown that cultivated groundnut possesses a narrow genetic base [9,10] due to a single hybridization event that occurred ~3500 years ago [11]. The genus *Arachis* has a total of nine sections possessing different genomes. Earlier reports have indicated the existence of a large range of variability among these sections. However, this variability cannot be exploited in a direct way because of ploidy or genome differences among the species [12,13]. In order to overcome the genetic bottleneck of restricted gene flow, the development of synthetic amphidiploids is an effective option to diversify the cultivated gene pool. To date, several synthetics have been developed by using different diploid species through colchicine-mediated genome duplication [14–17]. These highly diverse synthetics provide an opportunity for introgression of some important traits to cultivated germplasm. However, limited success has been achieved so far in using the wild species as genetic resources for the development of resistant cultivars. Nevertheless, release of an Indian variety (GPBD 4) containing resistance to foliar diseases in chromosome segments from *Arachis cardenasii* is an example of success. GPBD 4 is an improved variety developed as a second cycle derivative of an interspecific cross and is grown in several states in India for its desirable traits such as foliar disease resistance and high yield. Because of its high levels of resistance, *A. cardenasii* Krapov. & W. C. Greg. is the most widely used wild species in groundnut breeding programs aimed at improving foliar disease resistance. However, it is always better to look for alternative sources of resistance in order to diversify the cultivated gene pool [4]. Realizing the great potential of synthetic amphidiploids for enhancing the richness of the gene pool, this study was undertaken to broaden the genetic base of cultivated groundnut by introgressing resistance genes into five cultivated genotypes. We report the development of diverse genetic materials in groundnut with potential for several genetic and breeding applications.

2. Materials and methods

Synthetic amphidiploids ISATGR 278-18 [ICG 8138 (*Arachis duranesis* Kaprov. & W. C. Greg.) × ICG 13160 (*Arachis batizocoi* Kaprov. & W. C. Greg.)] and ISATGR 5B [ICG 8960 (*Arachis magna* Kaprov., W. C. Greg. & C. E. Simpson) × ICG 8209 (*A. batizocoi* Kaprov. & W. C. Greg.)] with $2n = 2x = 40$ were generated at ICRISAT (Hyderabad, India). Seeds from these amphidiploids were planted in a glasshouse at the University of Agricultural Sciences (UAS), Dharwad, India. Both amphidiploids were used to generate backcross populations with five elite varieties/genotypes, namely ICGV 91114, ICGS 76, ICGV 91278, JL 24, and DH 86 after making two backcrosses.

Flowers of cultivated genotypes were emasculated a day before pollination. Cross pollination was carried out before 10:00 a.m. on the following day by using the synthetic amphidiploids as pollen parents. Cotton swabs impregnated with gibberellic acid (GA_3) (0.5 mL; 75 mg L^{-1}) were wrapped around the base of pollinated pistils. Flowering was generally observed on recurrent parents about 45 days after sowing (DAS) and continued, allowing crossing for the next 30 days. The pods were harvested and percentages of crossed pods were calculated. In the next season, the F_1 plants were used as pollen parents for the first backcross to each recurrent parent. Pods of BC_1F_1 generation from all crosses were harvested and grown in the next season. These plants were then used to make second backcrosses. The BC_2F_1s were grown and selfed thrice to produce BC_2F_4 population after three seasons (Fig. 1).

Both amphidiploids were evaluated for component traits of rust and late leaf spot (LLS) resistances using a detached leaf technique [18]. On the 40th DAS, tetrafoliate leaves were excised from the pulvinous regions and arranged in plastic trays containing autoclaved sand in a randomized block design with two replications. In order to compare the disease