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Research Paper

Inheritance of morphological characters in Isabgol (*Plantago ovata* Forsk.)

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ABSTRACT

Isabgol (*Plantago ovata* Forsk.) is an important export oriented medicinal crop cultivated in India. Morphological characters are useful in crop improvement program as markers. However, there were no reports on inheritance of morphological characters in Isabgol. Our aim is to study the inheritance of leaf color (light and dark green), leaf curling (curly and normal), leaf hairiness (glossy and hairy) and plant height (tall and dwarf) using F_2 population derived from a cross derived from DPO-401 and DPO-324 parents. Hybrid (F_1) had light green leaves, no leaf curling, hairy leaf and tall plant type. F_2 segregation analysis revealed that, there were two (Lr_1 and Lr_2) genes with duplicate gene action controlling leaf color, single recessive gene (*cl*) controlling leaf curliness and two genes (H_1 and H_2) controlling the leaf hairiness with single dominant gene either of H_1 or H_2 producing the leaf hairy phenotype. Plant height was controlled by two genes (Dw_1 and Dw_2) with single dominant gene either of Dw_1 or Dw_2 producing the tall phenotype. The expression of these visible markers is independent of environment and therefore, can be useful as markers in linkage map construction and also as phenotypic tags in marker assisted selection.

Keywords: Medicinal plant, Isabgol, *Plantago ovata*, morphological characters, Inheritance, mutants.

Ponnuchamy Manivel, Harshal B. Deore and Rama Reddy Nagaraja Reddy*

ICAR-Directorate of Medicinal and Aromatic Plants Research, Boriavi, Anand-387 310, Gujarat, India.

*Corresponding author. E-mail: gpbreddy@gmail.com. Tel: +91-2692-271605/271606 –Ext. 201; Fax: +91-2692-271601.

INTRODUCTION

Isabgol (*Plantago ovata* Forsk.) is an important medicinal plant commercially cultivated in India and being exported in the world market. India holds monopoly in the trade of Isabgol seed and husk. It is a Rabi season crop, usually matures in 120 to 140 days after sowing. The seed husk, the seed epidermis having muco-polysaccharide layers are responsible for medicinal properties and are widely used against constipation, diarrhoea and intestinal irritation. The swelling property of the mucilaginous polysaccharide of husk is responsible for the medicinal property. It is hydrophilic in nature and upon absorbing water, increases by 10 times in volume.

Consequently, it is a very good dietary fibre, which stimulates peristalsis and helps in bowel clearance. The husk, which is about 25 to 30% of the seed, has the property of absorbing and retaining water and hence, it works as an anti-diarrheal drug. The husk is also used in calico printing, dyeing, agar-agar media preparation, gum and jelly making, as binder in tablets, as thickner and a fixative in ice-cream, confectionary and in cosmetics industries (Dhar et al., 2011). It has been used as a deflocculant in paper and textile manufacturing, as an emulsifying agent, as a binder or lubricant in meat products and as a replacement of fat in low-calorie foods (Dhar et al., 2011). Isabgol is largely cultivated in parts of Gujarat, Rajasthan and Madhya Pradesh. Sporadic area under cultivation is also found in the parts of Uttar Pradesh and Haryana.

Genus, *Plantago* exhibits a great deal of variation in breeding system. The out-crossing rates vary from 0 to 100% (Wolf et al., 1988). Very few species are self pollinated, however, *P. ovata* practices a blend of the two pollinating systems; genus includes both endogamous and allogamous (Sharma et al., 1993) in some plants of *P. ovata* synchrony in the receptivity of stigma and dehiscence of anthers help in the accomplishment of self pollination while others are cross-pollinated due to protogyny nature.

Being an introduced crop from the Mediterranean region, the major research issue is inadequate availability of genetic variability in Isabgol. Low genetic variability and lack of knowledge on inheritance of various qualitative and quantitative traits are some of impediments to the improvement of Isabgol. Hence, in this direction, a mutation breeding program was initiated at the ICAR-Directorate of Medicinal and Aromatic Plants Research (ICAR-DMAPR), Anand, Gujarat and many stable mutants with distinct morphological characters were developed. Mutant with distinct morphological characters such as leaf colour (DPO 259), hairiness (DPO 401), short leaf (DPO 276), curly leaf (DPO113), decumbent growth habit (DPO 395-3), earliness (DPO-14) and inflorescence types (DPO 9) were developed (Manivel and Saravanan, 2010, 2014).

Distinct, stable and uniform morphological characters will be useful as markers for identification of genotypes and also help in indirect selection of desirable phenotypes thus, quick improvement of Isabgol. Such markers can be placed on the linkage maps thus, genetic map saturation. However, for construction of linkage maps, a sufficiently large number of distinct qualitative traits are required (Reddy et al., 2012).

The genus, *Plantago* (family: Plantaginaceae) comprises 200 species, of which 10 occur in India (Anonymous, 1969). Genetics of some morphological characters have been studied in *Plantago* species. Gynodioecy is an out-breeding mechanism where both male-sterile and hermaphrodite plants found were controlled by two duplicate dominant genes for hermaphroditism, and that the double recessive $ms_1ms_1ms_2ms_2$ *gene*, is male sterile in *Plantago lanceolata* (Ross, 1969).

In *P. ovata*, the inheritance of economic characters, such as number of panicles, panicle length, seed yield and swelling factor using diallel analysis of F_1 progenies from seven parents was reported (Singh and Lal, 2009). However, there were no reports on inheritance of morphological characters such as leaf color (light and dark green), leaf curling (curly and non-curly), leaf hairiness (glossy and hairy) and plant height (tall and dwarf) in isabgol. In the view of the importance of these characters in Isabgol breeding, an attempt was made to study their inheritance using a bi-parental cross.

MATERIALS AND METHODS

The experimental material consisted of two mutants DPO-401 and DPO-324, and 1069 F_2 populations of cross DPO-401 × DPO-324. The genotypes, DPO-401 and DPO-324 are induced mutants of GI-2 developed in the project entitled "Creation of variability for the improvement of Isabgol (*P. ovata*)" at the ICAR-Directorate of Medicinal and Aromatic Plants Research (DMAPR), Anand, Gujarat. The mutant, DPO-401 had light leaf color, curly leaves, hairy and tall. In contrast, the mutant DPO-324 had dark green leaves, non-curly leaves (normal), non-hairy (normal) and dwarf phenotype. Crossing was affected during *rabi*, 2014 and F_1 plants were raised in 2015 at the research farm, the ICAR-DMAPR, Anand, India and were observed for the character of interest. Each of F_1 plants were selfed and harvested individually to raise the corresponding F_2 population during Rabi, 2016. Observations were recorded on color of leaf (light or dark) at flowering, shape of leaf (curling or normal) at flowering, hairs of leaf (hairy or normal) at flowering and plant height (tall or dwarf) at the time of harvesting. The data were subjected to chi-square analysis. The expected and observed frequencies were compared.

RESULTS AND DISCUSSION

Leaf color

Understanding the mode of inheritance of leaf color is critical for plant breeders to select appropriate parents, predict progeny performance, estimate breeding population size needed and increase breeding efficiencies. Several leaf color mutants were reported (Manivel and Sarvanan, 2014) in Isabgol.

The F_1 plant derived from the cross between DPO-401 and DPO-324 had light green color, indicating dominant nature of light green leaves in Isabgol. Out of 1,069 F_2 plant population, 1,006 showed light green, while 63 were dark green types (Table 1 and Figure 1); thus, the chi-square test indicated a good fit of 15:1 ratio indicating two (Lr_1 and Lr_2) gene epistasis with duplicate gene action. Leaf color is governed by two completely dominant genes, which produces the light green phenotype when genes are alone or together. The contrasting phenotype was produced when the Lr_1 and Lr_2 genes are in homozygous recessive condition. This is the first report on inheritance of leaf color in Isabgol. Variation in the leaf color is due to differences in the chlorophyll content. Such mutants are known as chlorophyll deficient reported in maize (Greene et al., 1988), rice (Hu et al., 1981) and soybean (Ghirardi and Melis, 1988).

Terao et al. (1985) reported that mutations in the leaf-color influence chlorophyll biosynthesis and degradation as well as, chloroplast development. Such mutants are ideal plant materials to investigate molecular mechanisms that underlie chlorophyll metabolism. Furthermore, leaf color genes could be used to improve seed yield because it can be easily scored and expressed at all developmental stages.

Curly leaf

Leaves, as lateral organs and determinants of growth

Table 1: Segregation ratios of qualitative characters observed in Isabgol Plantago ovata.

Cross (P1×P2)	Population	Character	Cross type (P1×P2)	F_1 Phenotype	F_2 Phenotype[$]		Total	Chi-square[+]	P- value
DPO-401× DPO 324	F_2	Leaf colour (*Lr*)	Light green × Dark green	Light green	Light green 1006 (1002)	Dark green 63(67)	1069	0.25(15:1)	0.70-0.90
		Curly leaf (*Lc*)	Curly leaf × Normal	Normal	Normal 932(802)	Curly 137(267)	1069	0.13(3:1)	0.70-0.90
		Leaf hairiness (*H*)	Hairy × Glossy	Hairy	Hairy 580(601)	Glossy 489(468)	1069	1.67(9:7)	0.10-0.20
		Plant height (*Dw*)	Tall × Dwarf	Tall	Tall 572(601)	Dwarf 497(468)	1069	3.50(9:7)	0.05-0.10

[$] Expected number of F_2 plants is given in parentheses. [+]Value in parentheses denotes segregation ratio.

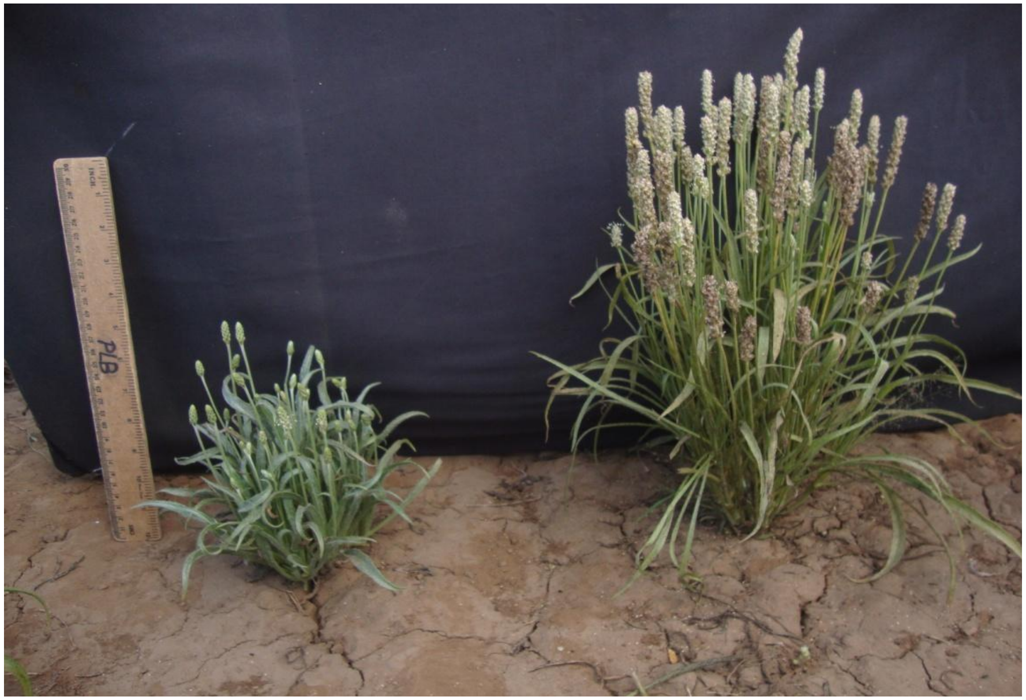

Figure 1: Leaves and plant phenotype of DPO-401 and DPO-324 mutants used to study the inheritance of leaf and plant type characters in Isabgol (*Plantago ovata*). DPO-401 (Dwarf, leaves are hairy, curly and dark green), while DPO-324 (Tall, leaves are non-hairy, normal and light green).

develop from flanking regions of the shoot apical meristem (SAM). Leaf shape, has a significant impact on yield, but information on inheritance of leaf shape is lacking in Isabgol. Manivel et al. (2010) discovered a random mutant with curly leaf shape in Isabgol. Its peculiarity is that the margin of the leaf curls upwards and inwards. The mutant cannot easily be distinguished from normal until eight weeks after germination, when its curly character is instantly visible from the normal leaf. The F_1 plant derived from the cross DPO-401 and DPO-324 had normal leaf, indicating the dominant nature of curly leaf shape. The F_2 analysis of 1,069 population was segregated out of which 932 F_2 plants had normal leaf, while 137 plants had curly leaf shape (Table 1 and Figure 1). The results fit into 3:1 ratio suggesting curly leaf is controlled by single recessive gene. The gene symbols, *CL* for normal and *cl* for curly leaf were proposed. Similarly, a single recessive gene (*cur*) controlling curly leaf shape was reported in peanut (Branch, 1987). In Arabidopsis, CURLY LEAF (CLF), gene controls cell division as well as, elongation of cells during expansion of leaf blade (Kim et al., 1998). Further, association of curly leaf shape phenotypes with plant growth and development needs to be investigated in Isabgol.

Leaf hairiness

Leaf hairs or Trichomes are cellular appendages that provide a physical barrier against insect pests for egg laying or larval movement, and thereby reduce plant damage. Leaf trichomes offers many advantages to plants such as conferring insect and disease resistance (Aruna et al., 2005; Satish et al., 2009) and reducing the rate of transpiration (Wu and Kao, 2009) and stress (Mehta et al., 2017). Presence of trichomes on the leaf surface hinders egg laying by shoot flies, thus, reducing the dead hearts in sorghum (Biradar et al., 1986). Trichome density is genetically controlled in plants (Satish et al., 2009). Mutants with hairiness were discovered for the first time in Isabgol (Manivel et al. 2010). There were no reports of inheritance of leaf hairiness in Isabgol. The plant, DPO-401 had hairy leaf type while the parent DPO-324 had normal leaf type. The F_1 exhibited hairy leaf type indicating dominance of the character. Further, F_2 analysis using 1,069 plants showed 548 with hairy and 528 with normal leaf (non-hairy) phenotype, thus, fitting the overall segregation into a good fit of 9:7 indicating two genes controlled with complementary gene action (Table 1 and Figure 1). There are two genes (H_1 and H_2) controlling the leaf hairiness in Isabgol. Single dominant gene either of H_1 or H_2 produces the leaf hairy phenotype. Hairy phenotype and genes could be used to improve yield and quality of Isabgol because the trait can be easily scored and expressed at all developmental stages. Furthermore, association of leaf hairiness with disease and pest resistance in Isabgol needs to be investigated.

Plant height

Plant height is an important trait that affects plant architecture. Dwarfism is one of the important agronomic traits that play part in increasing yield and quality of crop plants (Sasaki et al., 2002). Several mutants with dwarfing phenotype were discovered in Isabgol (Manivel et al., 2010). The mutant, DPO-401 was dwarf in plant height while the mutant DPO-324 was tall. The F_1 hybrid of the cross between DPO-401 × DPO-324 is tall indicating the dominance of tall over dwarf phenotype. F_2 segregation analysis of 1,069 plants revealed that there were 572 tall plants and 472 short plants fitting in the ratio into a good fit of 9:7 indicating two genes controlled with complementary gene action (Table 1 and Figure 1). The genes (Dw_1 and Dw_2) control the dwarfness in Isabgol. Single dominant gene either of Dw_1 or Dw_2 produces the dwarfing phenotype. Dwarfing phenotype and dwarfing genes could be used to improve yield. This is the first report on inheritance of plant height in Isabgol.

Conclusion

Inheritance of leaf color (*Lc*), curly leaf (*Cl*), leaf hairiness (*H*) and plant height (*Dw*) was determined for the first time in Isabgol (*Plantago ovata*). Expression of these traits was independent of environment and therefore, it can be incorporated effectively as markers in linkage map and can be effectively used as phenotypic tags in marker assisted selection. They also aid in identification of cultivars for their distinctness, uniformity and stability (DUS) in the era of plant variety protection and plant breeder's rights.

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