FLOWER AND SEED COLOURS AS MARKERS FOR NEW DERIVATIVES OF SIRATRO (PHASEOLUS ATROPURPUREUS)

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ABSTRACT

Pink and white flower mutants in P. atropurpureus *are described for the first time. The white flower arose spontaneously in one cross and in three different field populations of siratro in Queensland, and the pink flower was a result of irradiation. Both appear to be simple recessives. Foliage and seed characters of the pink-flowered plants are similar to those of siratro. All white-flowered plants lack the purple pigmentation of siratro, and their foliage and developing pods are light green. The seed of two siratro white-flower mutants are small, creamy green, and have thin seed coats, but a third mutant has uniform creamy brown seeds which are as large as, and have hard seed coats similar to those of siratro. The possibility of using pink-flower colour as a marker for an improved siratro appears to be remote as plants with the marker are agronomically inferior to siratro. Some white-flowered selected lines, whilst being marginally more vigorous than siratro, have relatively low seed yields and lack hardseededness. It is concluded that attempts to introduce genetic markers into improved lines of siratro are of doubtful value, and a seed certification scheme will be necessary to ensure purity of any new cultivars.*

INTRODUCTION

Since its release, the tropical pasture legume siratro (Hutton 1962) has shown several deficiencies. These include low commercial seed yields due to pod shattering, a restricted growing season in some areas, and susceptibility to the fungus *Rhizoctonia solani* which causes leaf and stem damage. Over the last ten years a series of crosses between an improved selection of siratro and a number of Central American introductions of *P. atropurpureus* have been made, and by selection in their progeny, lines have been produced which appear to be superior to siratro. A suitable marker character was sought which could be incorporated into the new material and thus enable it to be distinguished from siratro. This paper describes the origin in *P. atropurpureus* of the genetic markers pink and white flower colour**, and gives comparative data on the characters of the white-flowered lines, including colour and weight of the seeds and pods.

THE PRODUCTION AND INHERITANCE OF THE PINK-FLOWER CHARACTER AND ITS USE AS A GENETIC MARKER

Lots of 40 to 50 grams of F_2 seed from the cross which produced siratro (Hutton 1962) were each given a different dosage of gamma irradiation in Canberra in 1959 from a cobalt 60 source. Only two of the seeds survived the highest irradiation dosage (150,000r) and in the M_2 progeny of one of them two plants (out of 52) were found to have pink rather than the normal dark purple flower colour. No mutants were found in progeny from lower levels of irradiation. An M_3 population of 80 plants was raised from each of the pink-flowered plants in 1963. The plants were generally very weak, but all developed pink flowers. Progeny of one pink-flowered plant were more vigorous than those of the other and the original plant

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** The authors would appreciate any reports from farmers or others who have observed white (or pink) flowers occurring in fields of siratro.

was backcrossed to a promising siratro selection (B25). Seven plants resulted from the cross and progeny of about 80 or 90 plants were raised from each, and observed for flower colour. The resulting segregation purple:pink flowers are in agreement with a ratio of 3:1 (Table 1) and the pink-flower character is therefore behaving as a simple recessive allele.

TABLE 1

Segregations for flower colour in the F_2 from a cross between pink and purple flowered lines of P. atropurpureus

Plant number	Totals		χ^2 (3 : 1)	Probability
	Purple	Pink		
20	65	18	0.49	.50–.30
21	56	21	0.21	.70–.50
22	60	21	0.04	.90–.80
23	55	27	2.75	.10–.05
24	74	23	0.09	.80–.70
25	57	20	0.04	.90–.80
26	69	23	—	Perfect fit
Total	436	153	0.30	.70–.50

None of the pink-flowered segregates were as vigorous as the siratro controls, but seed was kept from a number of them.

One of the more vigorous pink-flowered F_2 plants from the previous backcross was backcrossed again (second backcross) to B25. This was followed by two further backcrosses of pink-flowered plants selected for vigour in segregating generations to superior derivatives of siratro. In November 1967, the best 163 of the pink-flowered plants from the fourth backcross were transplanted to the field at Samford, south-east Queensland, and their vigour and other characters compared with siratro and improved lines derived from it over the main growing season. By the end of the season it was apparent that all the pink-flowered plants were markedly inferior to the controls in vigour and other agronomic characters. The first backcross was also crossed with F_1's between B25 and three promising central American introductions. A population of 1385 F_2 plants with siratro and other controls were transplanted into the field in November 1965 and observed over two summer seasons for vigour and other characters. Altogether 47 pink-flowered plants appeared in this population, but all were inferior to the purple-flowered plants.

THE APPEARANCE AND INHERITANCE OF THE WHITE-FLOWER CHARACTER AND ITS USE AS A GENETIC MARKER

White-flowered plants were first noted in the field at Samford in 1966 in an F_2 population from two related F_1's. One of these (C9) was between two Mexican ecotypes of *P. atropurpureus* with purple flowers, and the other (C10) between one of these F_1 plants and B25. Each F_2 population was raised from the bulked seed of five F_1 plants, and segregation of white:purple flowers was 9:191 and 26:247 for F_2's from C9 and C10 respectively. Agronomically the white-flowered plants were equal to siratro. In order to investigate the inheritance of the white-flower character further, an F_2 population of 60 to 70 plants was raised from each of the five F_1's from C9. Of these, only one F_2 population produced white flowered plants in the ratio of 43 white: 214 purple. Fortuitously, this was the F_1 plant of C9 used to produce C10. These results show that the white flower is recessive to purple but further research is needed to determine its pattern of inheritance.

As the white-flowered plants were as vigorous as siratro, it was decided to use pedigree selection methods in the segregating generations from C10. The selected F_3 and F_4 populations, each of about 400 plants, were grown in the field in the 1966-67 and 1967-68 seasons respectively, but the results showed that no improvement on the siratro controls had resulted. Another attempt was made to obtain superior white-flowered types by crossing three of the best F_3 selections with two of the most vigorous siratro derivatives which were available at the time. From sixteen F_1 plants of the four crosses made, a large F_2 was raised (this segregated 655 white : 1807 purple) but only one of these crosses was promising enough to take to the F_3 generation which was grown in the field in the 1968-69 season. Two of the best F_3 selections from this cross are now at the F_4 stage and are being grown at Samford. The results so far show that these F_4 lines are only marginally more vigorous than siratro.

NATURAL OCCURRENCE OF WHITE-FLOWERED PLANTS IN FIELDS OF SIRATRO

Three separate occurrences of white-flower mutants in this legume have been reported. The first was received early in 1970 from Mr. C. G. Wilkinson of Walkamin near Mareeba in north Queensland who found a white-flowered plant in a siratro seed crop in 1969. A further occurrence of a white-flowered type was noted in April 1970 by Mr. J. James in a seven year old stand of siratro on his property at Ormeau, near Southport, southern Queensland. On Mr. James' property it seems that a white-flower mutant had appeared a year or so before and a small area of plants with white flowers had resulted from the seed shed by the original plant. In April 1971, the Queensland Government Botanist received plant and seed samples of a white-flowered type from a siratro pasture on the property of Mr. R. M. McNaught of Lower Wonga near Gympie.

Seed from these three white-flower mutants of siratro has been made available to us, and a study has been commenced on the seed and other plant characters in populations of the different mutants. In experiments with single spaced plants, the ratings for dry matter yields (based on sample cuts) at the end of April 1971 gave a mean rating of 4.03 for the Mareeba mutant and 3.74 for the Ormeau mutant. By comparison, the mean rating of the siratro controls was 3.75 and that of the selected white-flowered line described earlier, was 4.19.

In all white-flowered plants the absence of the purple anthocyanin pigment is characteristic not only of the flowers but also of the foliage and developing pods. As a result, the plants are an obvious light green in colour. On the other hand, the foliage on the pink-flowered lines is dark green with a tinge of purple similar to that of the purple-flowered types.

A further series of crosses is necessary to determine if all white-flower mutants are genetically identical.

COMPARISON OF POD AND SEED CHARACTERS IN WHITE AND PURPLE-FLOWERED F_3 LINES

The F_3 population of C10 was used for a study of pod and seed characters. At seed harvest in autumn, the percentage of "non-shattered" pods on each plant was determined and dried pods without the seed were weighed. Seed samples from every plant were also weighed and the effect on germination of treatment with commercial concentrated sulphuric acid for 20 minutes was measured.

TABLE 2

Pod and seed characters in white and purple flowered F_3 plants

Flower colour	No. of lines	Mean % non-shattering	Mean weight 20 pods (grams)	Mean no. seeds/pod	Mean weight 100 seeds (grams)	Mean % germination after 7 days	
						Acid treated	Nil treated
White	228	9.06	2.94	13.30	0.77	Nil	82.55
Purple	33	3.24 *	3.81 *	13.05	1.29 *	75.35	28.10 *

* Differences significant at the $P < 0.001$ level.

It is evident from the results (Table 2) that there are significant differences between white and purple-flowered plants in their important pod and seed characters. There is a significantly ($P < 0.001$) higher percentage of "non-shattered" ripe pods in white than in purple-flowered plants which could be associated with the significantly ($P < 0.001$) lighter weight of the pods in the white-flowered plants. The pods of the white-flowered plants are thinner and probably under less tension when ripe.

There was no difference in the number of seeds per pod, but the seed size of the white-flowered types is only 60 per cent of that of the purple-flowered. The smaller seed size and weight of white-flowered lines is reflected in seed yields per plant. The best white-flowered selections have given 30 to 76 grams of seed per plant, whereas the best purple-flowered selections usually give 120 to 250 grams of seed per plant.

The germination results in Table 2 are of particular interest. These show that seed of white-flowered plants has a thin coat and germinates satisfactorily without scarification, but is destroyed by the sulphuric acid treatment necessary for good germination in seed of siratro and purple-flowered plants. Dead seed accounted for the loss of almost 18% in the germination of the seed from the white-flowered lines. In the purple-flowered lines there was a mean of only 1.8% dead seed, but they had a mean of 70% hard seed which was reduced to 20% by the acid treatment. Germination tests with seed of the Gympie and Ormeau white-flowered siratro mutants has given similar results to those presented in Table 2 for the F_3 lines. Seed of the pink flowered-types and the white-flowered mutant from Mareeba has a similar germination pattern to that of purple-flowered ones.

Seed colour of the Gympie and Ormeau white-flower mutants and their progeny and selected lines is a distinctive creamy green, but the Mareeba mutant, which is later flowering, tends to have uniform creamy brown seed which is as large as that of siratro. The purple-flowered lines, siratro, and the pink-flowered types all have seed which varies in colour from dark gray to black.

DISCUSSION AND CONCLUSION

Pink and white-flowered mutants in *P. atropurpureus* have not been recorded before in the literature, and have not been found in the extensive collection of Central American introductions of this legume. The results from this study indicate that a high dosage of gamma irradiation is required to induce the pink-flower mutant as it only occured in the highest treatment, viz. 150,000r. Whether lower dosages of other types of radiation or treatment with a less destructive chemical mutagen like ethyl-methyl-sulphonate (Ehrenberg, Gustafsson and Lundqvist 1961) would induce this mutant without the severe genetic effects on vigour merits further research. No white-flower mutants appeared in any of the gamma irradiation treatments, but they appeared spontaneously in one cross and in fields of siratro. It

could be assumed that in *P. atropurpureus* a very low frequency of mutation to the white-flower allele occurs at the purple-flower locus. Both mutations are recessive and appear to be single gene mutations.

It is apparent from this paper that only limited progress has been made to date in breeding lines superior to siratro with a distinctive pink or white flower colour. Least progress has been made with the pink-flower character in spite of four back-crosses to superior siratro derivatives. This may have been due to the detrimental effects of the high dosage of irradiation on quantitative characters which are linked to the pink-flower gene. The indications are that a large number of further back-crosses are needed to eliminate the undesirable characters from this mutant and produce a pink-flowered line equal to or better than siratro in vigour.

More progress has been made with the spontaneous white-flower mutant as it has a good genetic background. As the pasture areas sown to siratro are expanded, a series of additional white-flower mutants will no doubt be reported. Further breeding work with this mutant can be expected to give better lines than siratro, but relatively low seed yields may always be a problem in these. The results of our breeding program suggest, however, that greater agronomic progress can be made with purple than with white-flowered lines. Most white-flowered lines have thin seed coats which may lead to a shorter storage life for its seed than for that of siratro unless it is stored under controlled cold storage. In addition, untreated seed of these white-flowered lines germinates readily and is not likely to persist in the soil for as long as that of siratro or its purple-flowered derivatives, and this could adversely affect regeneration. Since the breeding of superior replacements for siratro with a white or pink flower colour is difficult to achieve, it is more logical to concentrate on the development of purple-flowered lines and certify any that are released.

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