SOME HYBRIDS OF VARIETIES OF WHITE CLOVER (*TRIFOLIUM REPENS* L.).

By ADELA G. ERITH, B.Sc., Ph.D.
(*The University, Reading.*)

The hybrids described in this communication were obtained by artificially crossing the following varieties and races of White Clover (*Trifolium repens* L.):

1. *T. repens* var. *purpureum* ♀ × *T. repens* var. *rubescens* ♂.
2. *T. repens* var. *rubescens* ♀ × *T. repens* var. *purpureum* ♂.
3. *T. repens* var. *sylvestre* race *giganteum* ♀ × *T. repens* var. *purpureum* ♂.
4. *T. repens* var. *purpureum* ♀ × *T. repens* var. *sylvestre* race *giganteum* ♂.

The nomenclature of the plants is that given in the author's monograph on White Clover (1).

In pollinating the flowers the following procedure was adopted. An inflorescence was selected and the immature terminal and older basal flowers removed. The remaining flowers, which were in a suitable state for pollination, were then thinned out for convenience in manipulation. In order that there should be no possibility of "selfing," each flower used was carefully emasculated, although, from the work of previous investigators (2), and from my own experiments (1), it has been shown that *T. repens* is nearly, or even completely self-sterile. The emasculated flowers were covered with a muslin bag for one or two days until the stigmas were receptive. The flowers of the plants used as the pollen parents had been previously covered with muslin bags while still unopened, to prevent contamination from foreign pollen.

The actual pollination was effected by means of narrow strips of filter paper, half an inch in width, folded longitudinally and pointed at the tip, as described by Ware (3). These are of such a size that they can be easily inserted into the keel of the flower, from which the pollen is removed on the rough surface of the paper and readily transferred to the stigma of the flower of the female parent.

The seeds obtained were sown in pots in a cool greenhouse in the spring but, as there was no sign of germination at the end of a month,

they were removed from the soil and examined when it was found that they were all "hard." Each seed was therefore gently scratched and resown, with the result that germination followed almost immediately. The seedlings were subsequently transplanted into the open and their characters observed.

HYBRID 1. *T. repens* var. *purpureum* ♀ × *T. repens* var. *rubescens* ♂.

The female parent, var. *purpureum*, has white flowers like those of Wild White Clover (*T. repens* var. *sylvestre*). The upper surfaces of the leaflets, however, are either uniformly reddish purple with a narrow green marginal zone 1·5 to 2 mm. wide, or are green with extensive purple spots, the lower surfaces being of a much duller purple colour, which is less uniformly distributed. The purple coloration is due to a pink anthocyan pigment dissolved in the cell sap of the epidermal cells; almost all those of the upper epidermis are thus coloured while only a few of the cells of the lower epidermis are tinted.

Like Wild White Clover, plants of var. *purpureum* are cyanophoric, *i.e.* they contain glucosides which yield hydrocyanic acid through the action of enzymes present in the plant tissues. The cyanophorism can be most readily determined by anaesthetising the plants with chloroform. This allows the enzymes to come into contact with the glucosides, thereby liberating hydrocyanic acid, which is detected by its action on prepared sodium picrate paper(4, 5). The latter, which is normally bright yellow becomes changed to a brick-red colour by hydrocyanic acid. In testing plants for the presence of cyanophoric glucosides it was found that the depth of colour of the picrate paper varied somewhat with the age of the leaf tested; in order to obtain comparative results, the fifth or sixth leaf from the apex of a stolon was used in these experiments.

The male parent, var. *rubescens*, is endemic in the Scilly Isles whence plants were obtained about three years ago and grown in the Agricultural Botanic Garden of the University of Reading. These, while resembling var. *sylvestre* in general characters, have rose-pink flowers and are acyanophoric.

The F_1 plants of the cross had rose-pink flowers like those of the male parent but they were not uniform with regard to leaf colour, 19 plants having green, and 16, purple leaves. Each plant was tested for its cyanophoric properties, and of the 35 plants, it was found that 32 were cyanophoric and 3 acyanophoric. There was no correlation between the colour of the leaves and cyanophorism, since 17 of the cyanophoric plants had green and 15 had purple leaves, while 2 acyanophoric plants were green leaved, the remaining one being purple leaved.

HYBRID 2. *T. repens* var. *rubescens* ♀ × *T. repens* var. *purpureum* ♂.

In this reciprocal cross 25 F_1 plants were obtained, 13 bearing white and 12 rose-pink flowers. Fourteen of the plants had green leaves and 11 were purple leaved. With regard to cyanophorism, 21 plants were cyanophoric and 4 acyanophoric. As in the F_1 plants of cross 1 there was no correlation between leaf colour and cyanophorism, neither was there any correlation between leaf and flower colour, since, of 13 white-flowered plants, 6 had purple and 7 had green leaves; while 5 plants with rose pink flowers were purple leaved and 7 green leaved.

HYBRID 3. *T. repens* var. *sylvestre* race *giganteum* ♀ × *T. repens* var. *purpureum* ♂.

The female parent, known as Lodi Clover, Italian White Clover, or Giant White Clover, is a *gigas* mutation of var. *sylvestre*, which it resembles in general botanical characters, differing only in its larger size, shorter duration of life, smaller number of stolons, and acyanophorism.

The F_1 plants were large like those of the female parent, but were not uniform with respect to leaf colour and cyanophorism. Seven of the plants bore purple leaves, 9 had green leaves, and of the 16 plants 10 were cyanophoric and 6 acyanophoric.

HYBRID 4. *T. repens* var. *purpureum* ♀ × *T. repens* var. *sylvestre* race *giganteum* ♂.

The F_1 plants of this reciprocal cross were similar in all respects to those of the preceding hybrid: 13 plants had purple leaves and 13 were green leaved, while 14 were cyanophoric and 12 acyanophoric.

There was no correlation between leaf colour and cyanophorism in the plants of either of these two hybrids.

CONCLUSIONS.

The results of these investigations refer to the F_1 generation of the several crosses, since the plants are self-sterile and cannot provide an F_2 generation.

Leaf colour (F_1 *generation*).

Green leaf × purple leaf and reciprocals.

	Total no. of plants	Green leaved	Purple leaved
Hybrid 1	35	19	16
,, 2	25	14	11
,, 3	16	9	7
,, 4	26	13	13
	102	55	47
		1·17 : 1	

On account of the difficulty in the manipulation of the flowers, the numbers of the F_1 generation are small: they proved, however, sufficient to indicate the nature of the factors concerned in the inheritance of leaf colour and flower colour respectively.

From a close approximation to a 1 : 1 ratio it is clear that a single factor difference exists between the normal green leaved and the purple leaved plants. The results may be explained on the assumption that the rare purple leaved character is due to a single dominant factor, the purple leaved plants being heterozygous: they may also be interpreted equally well by assuming the presence in the green leaved plant of a factor inhibiting the purple tint. Further investigations will determine which hypothesis is the correct one.

Flower colour (F_1 *generation*).

		Total no. of plants	Pink flowered	White flowered
Hybrid 1	white ♀ × pink ♂	35	35	0
„ 2	pink ♀ × white ♂	25	12	13

The results indicate a single factor difference between the pink and white flowered plants, pink being dominant. Though similar in colour of flower, the pink flowered parents used were different plants and evidently different genotypes: that used in hybrid 1 was homozygous pink, the other in hybrid 2 being heterozygous.

Cyanophoric character (F_1 *generation*).

The parents and all the F_1 plants were examined in regard to their cyanophoric property with the following results:

		Total plants	Cyanophoric	Acyanophoric
Hybrid 1	cyan. ♀ × acyan. ♂	35	32	3
„ 2	acyan. ♀ × cyan. ♂	25	21	4
„ 3	acyan. ♀ × cyan. ♂	16	10	6
„ 4	cyan. ♀ × acyan. ♂	26	14	12
		102	77	25

From the ratios observed in these F_1 hybrids, it is clear that the acyanophoric parent of hybrids 1 and 2 is of a different genetic constitution from that used in hybrids 3 and 4.

The numbers are too small to furnish satisfactory evidence for a Mendelian interpretation of the inheritance of the cyanophoric character; further investigations are in progress to obtain numbers which it is hoped will be sufficient to determine the factors governing the inheritance of this character.

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