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COMPARISON OF PHYTOHAEMAGGLUTININS IN WILD BEANS (*PHASEOLUS ABORIGINEUS*) AND IN COMMON BEANS (*PHASEOLUS VULGARIS*) AND THEIR INHERITANCE

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Abstract—Populations of wild beans (*Phaseolus aborigineus*) giving both positive and negative haemagglutination test were detected. The positive extracts had the same specificity as common bean (*Phaseolus vulgaris*) phytohaemagglutinin (PHA) and induced mitosis in human lymphocyte cultures. They behaved similarly to common bean extracts in immunoelectrophoresis. The genetic analysis pointed to a single dominant trait of inheritance of the PHA. From the occurrence of positive and mixed wild populations it is concluded that no significant selection pressure and, hence, no vital role exists for the bean agglutinins.

INTRODUCTION

THE HAEMAGGLUTINATING activity in the extracts of bean seeds (*Phaseolus vulgaris* L.) has been known since Landsteiner and Raubitschek published their original observations in 1908.[1] The number of plants containing phytohaemagglutinins (PHA) or lectins is very large;[2] some of them have been isolated and characterized as proteins.[3] The bean agglutinin is of special interest because it induces mitosis in cultured human lymphocytes.[4] Seeds with and without haemagglutinating activity can be detected in the same plant species[2] but little is known about the inheritance of the responsible factor or factors.

RESULTS

Four of the nine wild-growing populations of *Phaseolus aborigineus* studied consisted of mixed seeds giving both positive and negative haemagglutinating reactions, while the seeds of five populations were uniformly positive. The mixed populations were from N.W. Argentina and Bolivia. The frequency of negative individuals varied between 21 and 77 per cent in localities situated near each other. Among the observed descendants, we frequently found segregating lines in spite of the fact that *Ph. aborigineus* is principally a self-fertilizing plant species.

The results of the haemagglutination test performed with the F_1 and F_2 generations of crosses between PHA-positive and negative plants are included in Tables 2 and 3. The F_1 generation showed only positively reacting individuals whether positive or negative plants were used as the female parent. Only two-fold differences in agglutinating titres were observed between homozygous and heterozygous seeds and were not considered significant. In F_2 seeds a 3:1 segregation was evident (Table 3).

[1] K. LANDSTEINER and H. RAUBITSCHEK, *Centr. Bakteriol.* **45**, 660 (1908).
[2] J. TOBISHKA, *Die Phythämagglutinine*, Akademie Verlag, Berlin (1964).
[3] T. TAKAHASHI, P. RAMACHANDRAMURTHY and I. E. LIENER, *Biochem. Biophys. Acta* **133**, 123 (1967).
[4] P. NOWELL, *Cancer Res.* **20**, 462 (1960).

The capacity of the *Ph. aborigineus* extracts to agglutinate different blood samples was compared with that of *Ph. vulgaris* with the results presented in Table 4. The action of extracts from *Ph. aborigineus* on human leucocyte cultures is shown in Table 5. The results indicate that the wild bean extract has a mitosis-stimulating activity similar to that of bean PHA. The immunoelectrophoretic patterns observed, when extracts from different lines of *Ph. aborigineus* and *Ph. vulgaris* were tested with the same anti-serum, revealed between six

TABLE 1. HAEMAGGLUTINATING ACTIVITY OF SOME NATURAL POPULATIONS OF *Ph. aborigineaus* BURK

Origin of population	PHA reaction of tested seeds		Frequency of negative seeds (%)
Tarija, Bolivia	49	162	77·1
Yala, Jujuy, Argentina	123	43	25·9
Salta, Salta, Argentina	133	36	21·3
Escoipe, Salta, Argentina	60	124	67·4
Rio Bermejo, Salta, Argentina	35	0	0
Mitisus, Mérida, Venezuela	211	0	0
Mérida, Mérida, Venezuela	42	0	0
Las Delicias, Táchira, Venezuela	84	0	0
Bochalema, Santander, Colombia	23	0	0

TABLE 2. PHA REACTION OF F_1 GENERATION OF *Ph. aborigineus* BURK

Crosses	F_1 seeds	
	Tested	Positive reaction
(+)—Pure lines (−)—Pure lines	29	29
(−)—Pure lines (+)—Pure lines	12	12

TABLE 3. SEGREGATION OF THE PHA—REACTION IN F_2 *Ph. aborigineus* AND *Ph. vulgaris*

	F_2 seeds tested		Expected F_2 segregation (3:1 ratio)		χ^2
	+	−			
4 *Ph. ab.* 1–67 (−) 3 *Ph. ab.* 1–67 (+)	17	7	18	6	0·22
Mountaineer Half Runner (−) 12 *Ph. ab.* 1–67 (+)	26	10	27	9	0·18
Bolita Salteño (−) Balin de Albenga (+)	241	81	241·5	80·5	0·004
Bolita Salteño (−) Cornell—49–242 (+)	242	77	239·25	79·75	0·127
Mountaineer Half Runner (−) Negro Nicoyano (+)	302	111	309·75	103·25	0·775
Mountaineer Half Runner (−) Balin de Albenga (+)	30	6	27	9	1·33

TABLE 4. COMPARATIVE HAEMAGGLUTINATING ACTIVITY OF BEAN EXTRACTS

Type of blood (conc. 1%)	Activity of the extract			
	Ph. vulgaris var. Balin de Albenga	*Ph. vulgaris* var. Guaria negro	*Ph. aborigineus* Jujuy, Argentina	*Ph. aborigineus* Mérida, Venezuela
Human O	1:3·000	1:20·500	1:5·000	1:2·500
Human A	1:5·000	1:20·500	1:5·000	1:2·500
Human B	1:10·000	1:20·500	1:10·000	1:2·500
Cow	neg.	neg.	neg.	neg.
Trypsinated cow	1:40·000	1:80·000	1:40·000	1:20·000
Rabbit	1:10·000	1:80·000	1:20·000	1:5·000

The highest dilution of a 5% seed extract producing visible haemagglutination is indicated.

TABLE 5. MITOGENIC ACTION OF *Ph. aborigineus* AND *Ph. vulgaris*

Extract	Blastic cells (%)	Mitosis (%)
Ph. aborigineus	12	1·8
	16	2·0
Ph. vulgaris	16	3·0
	27	6·0
—	0	0

Two drops of a 1% extract in physiological saline were added to each culture tube.

and eleven precipitation lines. In the cases of haemagglutinin-positive extracts of either bean species, one or two of the precipitation lines could be stained with sudan black. This was not the case when non-haemagglutinating extracts had been used.

DISCUSSION

The results of Table 1 indicate that haemagglutinating activity can be detected in many but not in all seeds of *Phaseolus aborigineus*, in natural populations of agglutinin-containing plants and in mixed populations. It is noteworthy that no pure negative populations were detected.

The distribution of PHA-positive seeds in the generations following the crosses indicate a single dominant gene as responsible for the inheritance of this character. In the work of Schertz *et al.*[5] a similar trait was observed in the inheritance of the lectin in *Ph. lunatus*, which, however, is different from *Ph. vulgaris* PHA in as much as it acts specifically on human blood group A cells. Indication for an extragenic influence on the ratio of positive and negative offspring was also observed by these authors. Tobishka and Lhotecka[6] observed that fertilizers may cause a four-fold increase in haemagglutination activity of *Ph. vulgaris* seeds. Takahashi *et al.*[3] have called attention to the conspicuous differences in chemical composition and

[5] K. F. SCHERTZ, W. JURGELSKY and W. C. BOYD, *Proc. Natl Acad. Sci. U.S.* **46**, 529 (1960).
[6] J. TOBISHKA and A. LHOTECKA-BRAZDOVA, *Z. Immunitätsforsch.* **119**, 225 (1960).

properties between the agglutinins from different varieties of *Ph. vulgaris*. In the PHA-negative strains, some inactive form of the agglutinin molecule may exist. This problem is presently under investigation.

In a previous study it was shown that extracts from *Ph. vulgaris* agglutinate the erythrocytes from all thirty-eight different blood samples tested, the only exception being cow-blood cells; these may be rendered susceptible by previous treatment with trypsin. This behaviour was not shared by any other agglutinin studied at that time.[7] The action of *Ph. aborigineus* extracts on cow blood was identical in this respect to that of *Ph. vulgaris*. *Ph. aborigineus* extracts also stimulated blastic transformation and mitosis in human lymphocytes, as does *Ph. vulgaris* PHA (Table 5).

An indication for the chemical similarity between the agglutinins of these bean species comes from immunoelectrophoresis. When sudan black coloration is applied, which has been shown to be characteristic for the kidney bean agglutinin,[8] identical precipitation lines can be detected with haemagglutinating extracts from any one of the two bean species when tested with rabbit anti-*Ph. vulgaris* serum but not with PHA negative extracts. This is in agreement with the view that they correspond to the agglutinins. Our observations thus confirm results of Kloz *et al.*[9] who demonstrated by quantitative immunological precipitation and by comparative immunoelectrophoresis the similarity of the proteins from *Ph. vulgaris* and *Ph. aborigineus*. They give new support to the close relationship between the two kinds of beans, which had been postulated on the basis of genetic tests,[10] immunological analysis[9] and graft affinity.[11]

The detection of populations of *Ph. aborigineus* with and without PHA not only made possible the experiments on inheritance of this factor, but also pointed to the possibility of relating modern strains of *Ph. vulgaris*, in which PHA-positive and negative varieties exist, to certain wild populations of *Ph. aborigineus*. The corresponding analysis indicated that the lack of agglutinating activity has to be considered as a spontaneously occurring loss mutation, resulting in negative reacting plants both among the wild beans (*Ph. aborigineus*) as, independently, among several cultivated varieties of *Ph. vulgaris*.[12]

The existence of factors with so spectacular biological activity as that of the agglutinins raises the question of their possible function within the plant. As they have a specific affinity to some carbohydrate material, Ensgraber[13] suggested that they may serve as carbohydrate fixers and be used to transport and store carbohydrate material. The fact that PHA-positive and mixed populations can be found growing wild would indicate that no significant selection pressure in favour of one of these genetic forms exists, and this would not support the view that they play any important role in the vital processes of the plant.

EXPERIMENTAL

The seed material used was part of the bean collection at the Genetical Section of the Faculty of Sciences of this University. Pure lines were produced, checking the progeny from single mother plants on homozygosity for agglutinating acitivity for three generations and were used for the production of the crosses. The seed production in the F_2 generation of *Phaseolus aborigineus* was very poor. Therefore, similar experiments were performed with different cultivars of *Ph. vulgaris* for the study of the segregation pattern.

[7] W. G. JAFFÉ, M. MONTBRUN, A. CALLEJAS and M. JAFFÉ, *Z. Immunitätsforsch.* **129**, 196 (1965).
[8] A. PALOZZO and W. G. JAFFÉ, *Phytochem.* **8**, 1255 (1969).
[9] J. KLOZ, E. KLOZOVÁ and V. TURKOVÁ, *Biol. Plant. (Praha)* **8**, 187 (1966).
[10] A. BURKART and H. BRÜCHER, *Der Züchter* **23**, 65 (1953).
[11] J. KLOZ and V. TURKOVÁ, *Serol. Museum Bull.* **29**, 8 (1963).
[12] O. BRÜCHER, *Proc. Trop. Region Am. Soc. Hort. Sci.* **12**, in press (1968).
[13] A. ENSGRABER, *Ber. Deutsch. Botan. Ges.* **71**, 349 (1958).

For the haemagglutination tests, single seeds were ground in a mortar and weighed portions extracted with ten times the amount of 1% NaCl solution overnight. Serial dilutions of these extracts were prepared and aliquots added to 0·5% suspensions of washed red blood cells. 1 per cent suspensions were used in the experiment of Table 4. If not otherwise stated, cells from rabbit blood were used. Cow-blood cells were treated for 1 hr with a 0·1% solution of crystallized trypsin, washed three times and suspended. Haemagglutination was observed after standing for 30 min at room temperature.

The action of the seed extracts on leucocyte culture was investigated by the technique of Favier *et al.*[14] Immunoelectrophoresis was performed on microscale using antisera of rabbits sensitized by injecting extracts from *Ph. vulgaris*.

[14] Y. Favier, M. Viette and M. Saint-Paul, *Nouv. Rev. Franc. Hématol.* **1**, 876 (1961).