Gene Transfer Between Tepary and Common Beans[1]

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Introduction
Successful crop production in desert regions requires adequate supplies of irrigation water. Vast amounts of capital, energy and labor must be committed to fulfill the water requirements of most crops. Whereas technological advances have better enabled water conservation during its conveyance and application, limited success has been achieved in improving plant drought tolerance. Improved drought tolerance of crop plants could result in a diminished consumptive demand for irrigation water. Many of the putative individual traits thought to be capable of imparting drought tolerance to crop plants have not been proven to be causally related to improved field performance. Possibly, by first investigating the regulation of water usage by related species that are indigenous to desert environments, one can ascertain the characters necessary for the survival of cultivated plants under low moisture conditions. It may then be possible to transfer beneficial characters from the xerophytic species to the commercial crop species which are not now adapted to arid environments.

The desert adapted tepary bean (P. acutifolius) and the drought susceptible common bean (P. vulgaris) offer a prospective system with which to make such a transfer. Common beans are an important dietary constituent of many people, and they have long been the subject of considerable research by plant scientists. Their adaptation to desert regions is quite poor however (Freeman, 1918; Thomas et al., 1983). Though not cultivated extensively today, tepary beans offer great potential for the improvement of P. vulgaris (Thomas et al., 1983). If tepary and common beans were easily cross-compatible, a combination of characters might be brought together capable of completely transforming the biological efficiency of common beans in arid regions. Unfortunately, a substantial degree of reproductive affinity does not exist among the species within the genus Phaseolus (Smartt, 1979). Rather, great effort must be expended to overcome the reproductive barriers that restrict gene-flow between them (Smartt, 1979; 1981). This situation is in contrast with the cereals, wherein several major crop species have been improved by the use of their extensive secondary gene pools (Harlan and de Wet, 1971; Feldman and Sears, 1981).

The classification of Harland and de Wet (1971) assigns related races or species to either the primary, secondary or tertiary gene pools of a crop species on the basis of reproductive affinity. Races or "species" which hybridize freely with the domesticated race belong to its primary gene pool. Species within the secondary gene pool are those which will cross with the crop species, but only after sometimes formidable interspecific barriers are overcome. Hybrids produced from such crosses are generally weak or sterile and the recovery of desirable types in advanced generations may be difficult (Harlan and de Wet, 1971). The tertiary gene pool involves even greater barriers to hybridization. Species contained within a tertiary gene pool often display either lethal or completely sterile hybrids when crossed with the cultivated species. More drastic measures such as embryo culture, grafting, doubling of the chromosomes, or the use of "bridging" species (species with greater affinity to the parents than they have with each other) to secure an interspecific hybrid are required (Harlan and de Wet, 1971).

Whereas a partial examination of the gene pools of P. vulgaris has been made (Smartt, 1981), a comprehensive description is not yet available. This is understandable since there are many related species within the genus Phaseolus (Marechal, 1978). However, it is clear that of the other domesticated species in that genus, P. coccineus (runner bean), P. lunatus (lima bean) and P. acutifolius (tepary bean), only P. coccineus is clearly a member of the secondary gene pool of P. vulgaris (Al Yasiri and Coyne, 1966; Smartt, 1981).

Although tepary beans, both domesticated and wild, are in the same subgeneric group as the common bean (Buhrow, 1983), difficulties in hybridizing the tepary bean with the common bean suggest that these species rarely fulfill any criteria warranting their inclusion in the secondary gene pool. As data presented below will indicate, P. acutifolius should be assigned to the tertiary gene pool of P. vulgaris.

The desirability of transferring certain characters of tepary bean to the common bean, such as drought tolerance, high temperature toler-

Figure 1. An interspecific hybrid ['Masterpiece' x F_1 (P.I. 440 x P.a. var. acutifolius 'MBAC' showing the phenotype similar to that of the "cripple" disorder. The initial cross was performed on March 13, 1931. Three embryos were excised and cultured 21 days after pollination and only one produced a viable plant. This hybrid flowered poorly and after 23 attempts to backcross it, during a five month period, it remained completely sterile.

ance, and pest and disease resistance, have been discussed by several authors (Mok et al., 1978; Waines, 1978; CIAT, 1979; Thomas and Waines, 1980; Prendota et al., 1982; Thomas et al., 1983). However, since tepary bean belongs to the tertiary gene pool, conveyance of such traits to P. vulgaris is indeed a tenuous proposition. In addition, the heritability of such traits as heat and drought tolerance is not known. It is likely that these traits are controlled by the interaction of many genes. The recent reports of fertile backcross lines, derived from P. vulgaris x P. acutifolius (and reciprocal) hybrids, are certainly encouraging (Thomas and Waines, 1982; Pratt et al., 1983). The recovery, however, of fertile backcross lines (P. vulgaris as recurrent parent) displaying desirable tepary characters as well, remains to be demonstrated convincingly.

As stated by Smartt (1979a), interspecific hybridization "is an enterprise beset with pitfalls." To help avoid these pitfalls, a review of research findings from many investigators, a discussion of how reproductive isolating barriers are essential to speciation, and how certain of these barriers prevent gene transfer between tepary and common beans are presented here. Strategies for successful gene transfer between tepary and common beans will be presented which hopefully will provide greater insight toward the achievement of an ultimate goal, that goal being the combination of desirable characters from an important world crop (the common bean), with those of the tepary bean, which is remarkably adapted to desert environments (Freeman, 1918; Nabhan and Felger, 1978).

Speciation and Genetic Isolation

The process of speciation occurs as populations follow separate lines of descent from a common ancestral population. In a given environment, new characters of adaptive value gradually become fixed. During this evolutionary process, divergent populations wherein such genetic changes are being incorporated may eventually achieve sub-species status. Genetic continuity among such sub-species, and with the original population from which they have been derived, remains unsevered. However, when sympatric populations become so distinct from one another that reproductive barriers between them come into effect, they are then deemed to have achieved separate species ranking.

Effective reproductive isolating mechanisms include both pre-zygotic and post-zygotic phenomena. Many pre-zygotic barriers are of greater importance in nature and can be overcome by the plant breeder (Hadley and Openshaw, 1980). Artifical cross-pollination and fertilization between P. vulgaris and P. acutifolius is performed readily by conventional methods in the greenhouse, as described by Buishand (1956), hence pre-zygotic barriers do not pose a problem. However, there are post-zygotic mechanisms which prevent successful recovery of interspecific common bean x tepary hybrids (Rabakoarihanta et al., 1979; Alvarez et al., 1981).

Post-zygotic isolation barriers can be manifested at any point in the life cycle of the hybrid sporophytic generation from the first mitotic cleavage of the zygote through the generation of functional gametes, and even later on in subsequent generations (Stebbins, 1958).

Of all the barriers to interspecific hybridization, perhaps the most common form of incompatibility is incongruity existing between the genes and chromosomes of the parents, resulting in developmental abnormalities (Stebbins, 1977). The genes within interspecific embryo or endosperm tissue, which are not fully compatible, must direct the complex molecular events responsible for normal development and

Table 1. Identifiable* parental genotypes which have been success-fully used in hybridization programs between *P. vulgaris* and *P. acutifolius.*

P. vulgaris	*Investigator*
Great Northern	Honma (1956), Rabakoarihanta et al. (1978)
Gallatin 50	Rabakoarihanta et al. (1978)
Jacobs Cattle	Hwang (1979)
U.S.D.A. PI 316-912	Hwang (1979)
Masterpiece	Thomas and Waines (1980), Pratt et al. (1983)
F₁ (Pinto UI-114 x PI 319-441 (wild))	Thomas and Waines (1980)
F₁ (Porillo Sintetico x Puebla 152 BL)	Pratt et al. (1983)
F₁ (Jamapa x ExRico 23)	Pratt et al. (1983)
F₁ (Sanilac x Puebla 152 BL)	Pratt et al. (1983)
C.I.A.T. G03807	Prendota et al. (1982)
C.I.A.T. VEF 2068	Prendota et al. (1982)
C.I.A.T. G11822	Prendota et al. (1982)
Romano Pole	Nelson (1982)
Blue Lake Pole	Nelson (1982)

P. acutifolius	*Investigator*
U.S.D.A. PI 310-800	*Mok et al. (1978)*
U.S.D.A. PI 321-637	*Mok et al. (1978)*
U.S.D.A. PI 321-638	*Thomas and Waines (1980)*
U.S.D.A. PI 319-443	*Thomas and Waines (1980), Nelson (1982), Pratt et al. (1983)*
U.S.D.A. PI 319-444	*Nelson (1982)*
U.S.D.A. PI 406-633	*Nelson (1982)*
C.I.A.T. G03560	*Prendota et al. (1982)*
C.I.A.T. G 40005	*Prendota et al. (1982)*
F₁, F₂ (PI 440-790 x P. acutifolius var. acutifolius	*Pratt et al. (1983)*

P. acutifolius (wild)	*Investigator*
P. a. var. latifolius U.S.D.A. PI 319-446	Thomas and Waines (1980)

P. vulgaris (wild)	*Investigator*
U.S.D.A. PI 319-441	Thomas and Waines (1980)

*Identifiable refers to accessions that may be requested from plant collections by researchers vs. those accessions bearing the breeder's personal designations.

functioning of the hybrid embryo. Embryos which are the result of *P. vulgaris* x *P. acutifolius* crosses develop abnormally, due partially perhaps to the insufficient production of cytokinins (Nesling and Morris, 1975).

Interspecific Hybridization between Common and Tepary Beans

The breakdown and subsequent abortion of ovules in this cross generally occurs between 5 to 26 days after pollination (Al Yasiri and Coyne, 1964; Mok et al., 1978). The greatest degree of abortion is evident approximately 14 to 20 days after pollination (Al Yasiri and Coyne, 1964). While the production of mature seed in situ from this cross is possible (Smartt, 1979; Prendota et al., 1982), investigators usually resort to embryo rescue techniques since a much higher frequency of mature hybrids can be obtained.

Embryos are generally excised 14 to 24 days after pollination and cultured on a medium containing 30 to 40 g/l sucrose along with minerals, salts and vitamins (Honma, 1955; Mok et al., 1978; Alvarez et al., 1981; Pratt et al., 1983). Numerous studies have indicated that the

genotype and ontological development of the embryo have a greater role in determining its development in culture and the survival of subsequent plantlets than does the culture medium per se (Mok et al., 1978; Raghavan and Srivastava, 1982; Pratt et al., 1983; Thomas et al., 1983).

The interspecific hybrids from this cross display substantial phenotypic variability, based on morphological and growth characteristics. The first reported interspecific hybrids (Honma, 1955; 1956) were described as being intermediate between the parents in morphological characters, the plants becoming more like the *P. vulgaris* parent with time. Floral morphology was described as being similar to *P. vulgaris.* Subsequent reports of interspecific hybrids have indicated the morphological characters to be either abnormal or intermediate (Smartt, 1970; Alvarez et al., 1981; Prendota et al., 1982; Pratt et al., 1983). Investigators also have described the floral characters as resembling more nearly the tepary parent (Smartt, 1970; Prendota et al., 1982; Pratt et al., 1983).

Interspecific hybrids from this wide cross display substantial differences in growth. Smartt (1970) reported that *P. vulgaris* x *P. acutifolius* hybrids were more vigorous and persisted longer than the plants from the reciprocal cross. However, the opposite results have been observed (Prendota et al., 1982). Mok et al. (1978) reported that hybrids, which were obtained through embryo culture, grew vigorously at first but then essentially ceased growing. Honma (1955) described the opposite, stating that the hybrids were weak at first, but that "they appeared to be entirely normal" by the time they were 15 inches high. Prendota et al. (1982) observed two predominant types of hybrids. One was characterized by a "severely unbalanced" and stunted phenotype whereas the second included plants which are much more vigorous and well-balanced, but with the presence of viral like symptoms. The presence of such symptoms as leaf curling and sectoral chlorotic patterns have been frequently reported (Smartt, 1970; Mok et al., 1978; Alvarez et al., 1981; Pratt et al., 1983). The abnormal plants may resemble those of the "cripple" phenotype derived from certain intraspecific *P. vulgaris* crosses (Coyne, 1965) (Figure 1).

A "limited number of seed" was reported to have been produced by each of the first four putative hybrids between these species (Honma, 1956). More recent investigations indicate that from a total of scores of putative interspecific hybrids, not one has yielded viable diploid seed. However, the interesting observation has been made that one sterile hybrid produced one seed per pod after an exogenous application of gibberillic acid (Nelson, 1982). I have observed hybrids which produced a few ovules suitable for in vitro culture; however, the seedlings collapsed not long after transfer to soil (Pratt et al., 1983). In light of the fact that essentially all putative hybrids from this cross are highly self-sterile, any reports of fertile hybrids should carry with them strong evidence of authenticity.

What accounts for the strong degree of sterility in these interspecific hybrids? If there is little homology between some or many of the chromosomes of the two parental species, successful pairing between all of the chromosomes may occur only very rarely. In the pollen mother cell (PMC) squashes (at metaphase I) thus far examined a mean of 6 to 8.7 bivalents (out of a possible eleven) have been reported (Hwang, 1979; Rabakoarihanta et al., 1980; Prendota et al., 1982). As many as 10 and even 11 bivalents have been detected, though their frequency is extremely low (less than 6% of the cells examined) (Rabakoarihanta et al., 1980; Prendota et al., 1982). The substantial variability in effective pairing between the chromosomes could suggest the involvement of genic factors in addition to some degree of chromosomal differentiation between the species. Meiotic phenomena which result in the formation of laggards and uneven chromosomal migration to the poles, observed in common bean x tepary (and reciprocal) hybrids (Rabakoarihanta et al., 1980; Prendota et al., 1982) can result in the development of abnormal daughter cells, and hence inviable gametes (Figure 2). Combined with the poor survival of dividing embroys (Rabakoarihanta et al., 1980; Prendota et al., 1982), these factors could result in the high degree of sterility displayed by the hybrids. This sterility presents a serious obstacle to the prospective transfer of useful characters from *P. acutifolius* to *P. vulgaris.*
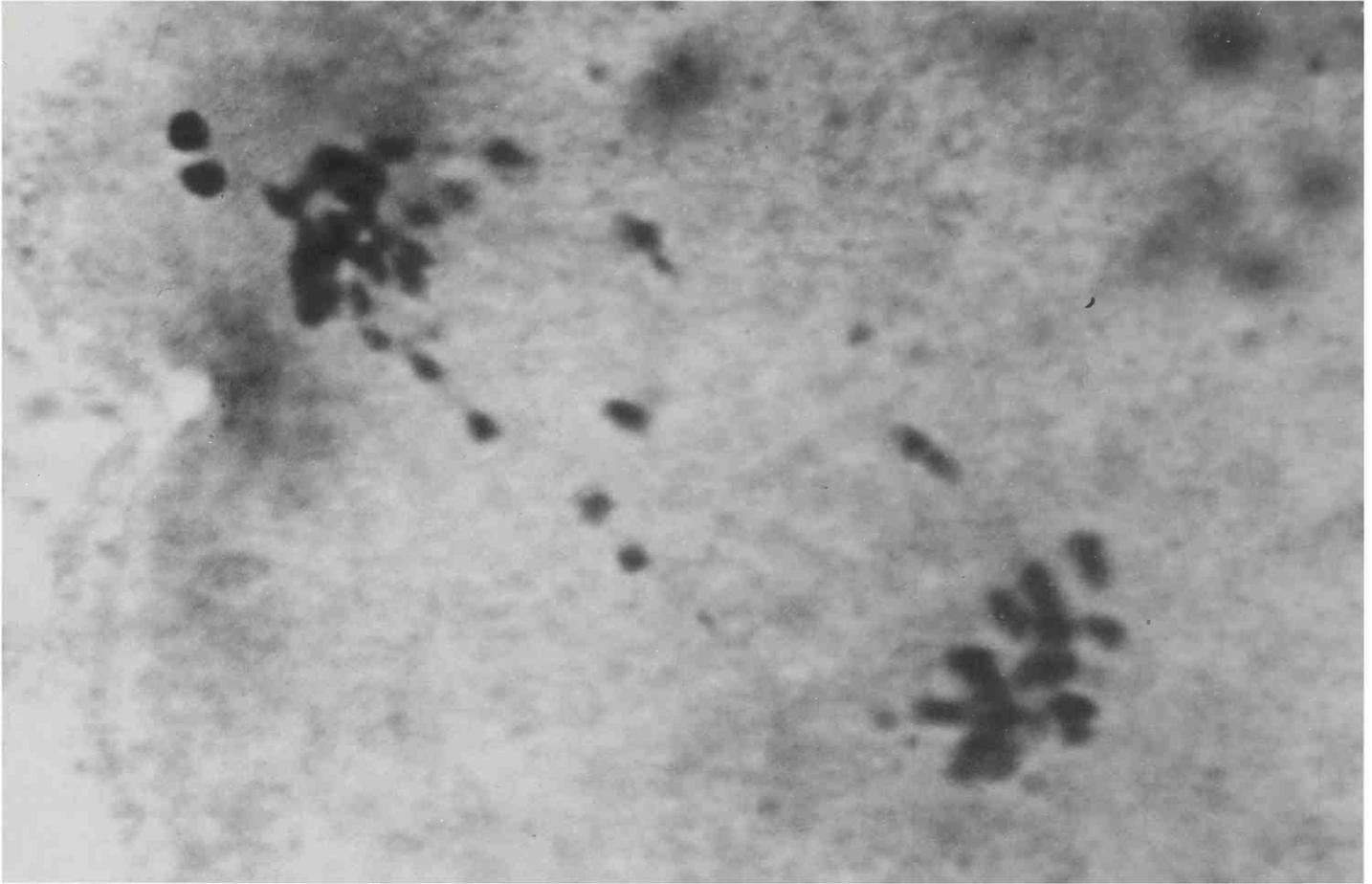
Figure 2. A PMC squash at late anaphase I obtained from the interspecific hybrid ['Masterpiece' x F₂(P.I. 440-790 x P. a. var. acutifolius 'MBAC')]. Note the presence of several laggards which are unlikely to reach the poles.

Strategies for Successful Gene Transfer

If a greater number of hybrids could be produced more readily, then the probability of obtaining more fertile genotypes would be enhanced. Sufficient research has now been performed with the common bean x tepary (and reciprocal) cross, that breeders can at least utilize parental accessions with the demonstrated ability to yield viable interspecific hybrids (see Table 1).

It is thought that the wild relatives of the tepary and common bean P. acutifolius var. latifolius and P. vulgaris (var. aborigineus), respectively, were the ancestral progenitors of the present cultivated varieties (Kaplan, 1965; Evans, 1976; Nabhan, 1978). If these species diverged from common ancestral populations in Central America, then, according to the concept of biological speciation, it may be valid to assume that the reproductive barriers between these wild species are less severe than those existing between their more diverged domesticated counterparts.

Gentry (1969) has posed the question whether or not the use of such wild, related species and varieties would enhance the probability of success for interspecific hybridization within the genus Phaseolus. Several independent groups have already utilized the wild species in such a manner, with considerable success (Thomas and Waines, 1980; Prendota et al., 1982; Pratt et al., 1983). The utilization of parents with enhanced "gametic diversity" (heterozygous and/or heterogeneous plants) also is a method of proven value in species crosses in the genus Phaseolus (Wall and York, 1959; Bemis and Kedar, 1961; Thomas and Waines, 1980; Nelson, 1982; Pratt et al., 1983). By crossing cultivars and land race accessions with related wild species one can obtain parents with a very high degree of "gametic diversity."

The use of such agents as colchicine to double the chromosome number of interspecific hybrids with the hope of improving fertility in subsequent backcross generations is also under investigation (Prendota et al., 1982; Thomas et al., 1983). Prendota et al. (1982) have doubled the chromosome complements of eight P. acutifolius x P.

vulgaris hybrids. From the completely sterile hybrids, colchicine induced tetraploids were obtained which set several mature seeds. While the potential for obtaining useful lines directly from such plants is doubtful, multivalent pairing in the allotetraploid has been observed (Prendota et al., 1982). The formation of such multivalents indicates that some degree of heterogenic exchanges may have occurred. This implies that as backcrossing is initiated with P. vulgaris (as the recurrent parent) the sterile triploid will contain some tepary genes. The crossover events, due to the previous intergenomal pairing within an individual allotetraploid, are unlikely to produce sufficient variability for the recovery of desirable multigenic tepary characters in the subsequent backcross progeny. However, such restricted crossing over may increase the probability of transferring 1 or 2 gene traits without the problem of "hybrid breakdown" due to a substantial amount of intergenomic crossing over (Stephens, 1961; Wall, 1970).

Attempts to obtain viable, fertile backcross plants have met with variable success. Smartt (1970) was unable to obtain backcross individuals and Mok et al. (1978), Thomas and Waines (1980) and Pratt et al. (1983) found it expedient to utilize embryo rescue to obtain backcross plants. Rabakoarihanta et al. (1980) encountered less difficulty when using the tepary as the backcross parent, whereas Thomas and Waines (1980) and Pratt et al. (1983) were successful in obtaining backcross seed utilizing the common bean as the recurrent (backcross) parent. Backcross individuals display great variability with respect to both vegetative phenotype and degree of fertility (Thomas and Waines, 1982; Pratt et al., 1983).

In addition to the desirable genes contributed by the tepary and its wild relatives, deleterious genes undoubtedly will be present in interspecific hybrids as well. The only possible method to eliminate them is by repeated backcrossing to a recurrent parent followed by selection. While the greatest strength of a backcross program is its ability to transfer one or several specific genes to a recurrent parent, it is possible to transfer quantitatively inherited traits also. Methods for such an

Figure 3. *A fertile F_1BC_2 plant grown under dryland conditions in the semi-arid climate of Arizona (1982). Such individuals provide the basis for continuing work with the common bean x tepary hybridization program.*

approach (with intraspecific hybrids) are discussed by Bliss (1980).

Transfer of multigenic traits between related species using a simple recurrent backcrossing system may be significantly restricted, however (Stephens, 1961). It appears that with interspecific *Phaseolus* crosses, reduced chiasmata formation (Maŕechal, 1971) and subsequent crossing over, due to structural or molecular differences between apparently homologous chromosomes will prevent the separation of linked genes. This result would be analogous to the consequences of a reduction in effective chromosome map length as discussed by Hanson (1958). This may make it extremely difficult to transfer all of the desired genes of a complex multigenic character in teparies to common beans without the transfer of undesirable ones (e.g., small seed size) which may be closely linked.

In common bean x tepary crosses (and reciprocal) as many as 3-5 of a total complement of 11 chromosomes in F_1 PMC squash preparations frequently have been observed to not pair (Hwang, 1979; Rabakoari-hanta et al., 1980; Prendota et al., 1982). Many of the genes conferring desirable agronomic traits may be located on the more non-homologous (homeologous) chromosomes of such crosses, between which crossing-over occurs at a very low frequency, if at all. Hence, the recovery of individuals which have acquired complex multigenic traits from *P. acutifolius* likely will occur at a very low frequency.

In addition to the problems encountered due to fewer cross-over events in a genome derived from two distinct species, apparently

Figure 4. *A fertile BC_2 plant obtained from a common bean x tepary hybrid after two backcrosses to P. vulgaris. Most BC_2 plants do not display such a high level of fertility. While this degree of fertility is encouraging, one must still ask whether or not sufficient P. acutifolious characters will be expressed in its progeny to allow the selection of desirable genotypes.*

Figure 5. A BC_3 plant showing truncate primary leaves (a tepary character) with a long petiole (a common bean character). Above that leaf, a primary leaf from the recurrent parent P. vulgaris 'Sanilac' which displays the cordate morphology. This phenotype was not observed in the BC_1 or BC_2 generations yet appeared in the BC_3 generation.

non-random cross-over events and differential gametic or zygotic elimination may occur which actively excludes the genes contributed by the other species from subsequent generations (Stephens, 1961; Wall, 1970). Recombination apparently has an optimal level in certain crosses. Wall (1970) concluded from studies with P. coccineus that gene transfer is facilitated by mating systems which achieve an optimum balance between enhanced heterospecific genome recombination and those deleterious effects (e.g. duplications and deficiencies) of recombination which result in "hybrid breakdown." To achieve this balance it was recommended that a backcross program include alternate cycles of sib-mating (Wall, 1970).

This author's attempts at sib-mating among BC_1 (Back Cross) plants derived through the interspecific hybridization of P. vulgaris x P. acutifolius have not been encouraging. Only weak individuals have been obtained to date. Significant variability exists among the BC_2 populations. It is with these populations (as large as possible) that sib-mating, followed by several generations of selfing, could perhaps result in the necessary diversity of phenotypes from which to select individuals with desirable metrical characteristics e.g. drought tolerance, derived from the tepary parent. Crossing BC_1 with BC_2 plants also might aid in increasing the frequency of individuals with desirable genotypes if substantial elimination of P. acutifolius genes has occurred by the BC_2 generation. The problem of restoring fertility without loss of sufficient tepary genes to produce individuals with desirable tepary characters will present a formidable challenge, though not an insurmountable one (Figures 3 and 4).

Authentication of Interspecific Hybrids

The utilization of diverse tepary and common bean parents presents various problems to the breeder. When grown in temperate environments, or at increasing latitudes, teparies may display irregularities in growth and development (Hendry, 1919) or flowering (Pratt and Erickson, 1982). The phenotype of tepary accessions may be highly variable due to environmental parameters or substantial heterogeneity within particular accessions. Hence, the use of morphological markers alone is insufficient evidence of hybridity. Smartt (1979a) discusses the difficulties involved when attempting to interpret primary leaf morphology as evidence of interspecific hybridization. The variable inheritance of this marker makes its use questionable (Figure 5). However, the lanceolate leaf character of wild P. acutifolius var. acutifolius has some potential for use as a marker in common bean x tepary crosses (Pratt et al., 1983). Other promising markers are those of flower bracteolet size, shape and color, and flower size and color, but all require further investigation.

Of course sterility is a good indicator that a wide cross has been made, however it is not a foolproof one. "Apparent sterility" of selfs may be due to a strong photoperiod requirement, abnormalities such as the "cripple" disorder, or perhaps to the expression of recessive male sterility. Smartt (1979a) pointed out that it is essential to compare putative hybrids with parental progeny (especially when utilizing intraspecific hybrids), rather than with the actual parents themselves.

When putative hybrids appear to be partially fertile, a cytological or biochemical examination is recommended. The frequency of pollen

mother cells displaying chromosomal abnormalities such as a lack of pairing at metaphase I, the presence of laggards at anaphase I, and the appearance of micronuclei in tetrads are far stronger evidence of hybridity than are morphological characters or "intuition." A word of caution is also necessary regarding the interpretation of cytological preparations. Marechal (1971) showed very clearly that asynchronous chromosomal migration during anaphase I and II in PMC figures can be attributed to environmental causes. An examination of other cytological work performed with *Phaseolus* also demonstrates that the presence of univalents in metaphase I and anaphase I and II PMC figures can occur in the parental species, likely due to environmental causes (Sarbhoy, 1978; Sinha and Roy, 1979). Hence, as evidence of hybridity, the examiner should provide data showing the frequency of such meiotic abnormalities in comparison with those of the parental species. Biochemical markers, such as Fraction I protein and isozyme banding patterns have not yet been utilized in evaluating this cross and should be invaluable to the breeder.

Conclusion

The barriers which once prevented successful interspecific hybridization between *P. vulgaris* and *P. acutifolius* are no longer insurmountable. Gene transfer between these species has now reached a second phase. The most productive avenue by which to fix desirable tepary characters in an acceptable common bean background is now being studied by several investigators. With the transfer of specific tepary characters to the common bean accomplished, researchers may then be able to ascertain the most valuable traits necessary for the improved drought tolerance or water use efficiency of common beans in desert environments. More heat tolerant, pest and disease resistant lines may also be produced from tepary x common bean (and reciprocal) crosses. Then perhaps, as suggested by Gentry (1969), modern plant scientists can start "returning the favor" to the native inhabitants of North America whose ancestors originally domesticated these valuable food crops.

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