Grafting as a means of alleviating the cripple phenotype in two- and three-species congruity backcross (CBC) *Phaseolus*

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Introduction

Cripples are symptomatic of hybrid breakdown due to incongruity. These occur at varying frequencies in wide crosses: intraspecific *Phaseolus vulgaris* crosses between centers of origin (Shii, et al., 1980) and interspecific crosses such as *P. coccineus x [P. vulgaris x P. acutifolius]* (Mickelson, 1992). The genetic control of crippling has been attributed to two dominant, complementary genes (*DL-1, DL-2*) in the F_1 (Shii, et al., 1980) and duplicate, recessive or dominant, cripple lethal (*L*) genes in the F_2 (Bokosi, et al., 1995). Cripples usually comprise a small portion of any generation. In one three-species CBC test cross (K5 x H15), 100% cripples were generated (Mickelson, 1992). These dwarf cripples would not respond to either vernalization (cold) or GA_3 treatments, remaining crippled until their eventual death after 1-2 years. While F_1 and F_2 cripples are of interest to geneticists, they represent evolutionary bottlenecks and prevent breeders from selecting favorable recombinants. As a means of circumventing crippled growth habit, we theorized that the crippled phenotype might be due to a root deficiency in cytokinin production. Crippling could be removed via grafting a crippled scion onto a healthy, non-crippled rootstock.

Materials and Methods

Healthy, noncrippled *P. vulgaris* 'Black Turtle Soup' (BTS) and 'Kentucky Wonder' (KYW) and crippled two- and three-species (including *P. vulgaris, P. acutifolius, P. coccineus*) CBC F_1 (93-64, 94-96) were used in this experiment. The following graft combinations were used: controls (BTS ungrafted, KYW ungrafted, BTS/BTS, KYW/KYW, BTS/KYW, KYW/BTS), crippled scions (93-64/BTS, 93-64/KYW, 94-96/BTS, 94-96/KYW), and crippled rootstocks (BTS/93-64, BTS/94-96, KYW/93-64, KYW/94-96). Either recently germinated seedlings or 1-2 year-old crippled plants were used. Side and splice grafting methods were employed. Traits measured for any changes in rootstocks or scions were survival/death (+/-), terminal leaflet length/width ratios, internode length, anthesis, crippled leaf phenotype, and yield (seed number, pod number, 100 seed weight).

Results and Discussion

Splice grafting was the easiest technique and it produced the highest percentage of graft unions that "took". Newly germinated crippled seedlings performed the best in the experiment (Table 1). Older crippled seedlings eventually died, although this was not due to a graft union problem. Leaf length/width ratios and internode lengths did not differ significantly between grafted/nongrafted and scion/rootstock (except for length/width ratio for 94-96; $P=0.05$). Two different 93-64 F_1 seedlings reverted from cripples to semi-normal, flowering individuals. Since these were the only genotypes to respond favorably to the grafting procedure, not all cripples are genotypically the same.

All yield data was highly significant ($P<0.001$) for rootstocks, scions, and crippling. This would be expected since most crippled scions did not revert to normals and, consequently, never flowered. Flowering scions that reverted to normal (93-64) produced seed, although the yield was significantly reduced. The grafting process clearly affects yield, although its effect is not always negative. For instance, BTS/BTS, BTS/KYW, 93-64/BTS, and BTS/93-64 graft combinations had a significant increase in 100 seed weight over BTS ungrafted plants. Thus, the crippled rootstocks cannot be assumed to be completely deficient in cytokinin production. There is some obvious transference of plant growth substances from the roots to the scions that aided crippled 93-64 scions reverting to a normal phenotype. F_2 populations derived from selfing these grafted "normal" 93-64 plants were completely normal, exhibiting neither visible symptoms of crippling nor abnormal seed protein electrophoretic profiles. It is doubtful that the genetic control of crippling is due to either dominant or recessive genes, since the F_2 populations did not display any cripples.

References

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Mickelson, HC. 1992. Congruity backcrossing as a method of establishing multi-species gene pools in Phaseolus. MS Thesis, Univ. Minn. St. Paul.

Shii, CR, MC Mok, SR Temple, and DWS Mok. 1980. Expression of developmental abnormalities in hybrids of Phaseolus vulgaris. J. Heredity 71:218-222.

Table 1.
Effect of scion age and grafting method on leaf length/width ratios and internode lengths of *Phaseolus vulgaris* 'Black Turtle Soup' (BTS) and 'Kentucky Wonder' (KYW) rootstocks and two- or three-species *Phaseolus* hybrid, crippled scions (93-64, 94-96).

				Scion				Rootstock			
Scion	Rootstock	Age[1]	Scion Graft Type[2]	Length (L) (cm)	Width (W) (cm)	L/W ratio	Internode length (cm)	Length (L) (cm)	Width (W) (cm)	L/W ratio	Internode length (cm)
BTS	--	0	0	6.2	5.5	1.13	1.3	6.2	5.5	1.13	1.3
BTS	BTS	1	2	4.2	3.0	1.4	2.8	-.-[3]	-.-	-.-	-.-
BTS	KYW	1	1	7.5	4.3	1.74	2.1	-.-	-.-	-.-	-.-
BTS	93-64	1	2	7.6	3.5	2.17	2.7	-.-	-.-	-.-	-.-
BTS	94-96	1	2	6.7	4.1	1.63	3.0	1.7	1.1	1.54	2.1
KYW	--	0	0	1.4	6.9	1.65	11.3	1.4	6.9	1.65	11.3
KYW	KYW	1	1	10.0	7.1	1.41	8.3	5.5	4.0	1.38	10.3
KYW	BTS	1	1	13.1	11.5	1.14	7.7	-.-	-.-	-.-	-.-
KYW	93-64	1	2	-.-	-.-	-.-	-.-	-.-	-.-	-.-	-.-
93-64	--	0	0	2.9	2.0	1.45	1.6	2.9	2.0	1.45	1.6
93-64	BTS	2	1	-.-	-.-	-.-	-.-	4.2	2.2	1.91	1.4
		1	2	7.0	3.0	2.33	7.2	-.-	-.-	-.-	-.-
93-64	KYW	2	1	-.-	-.-	-.-	-.-	3.9	3.4	1.15	1.4
		1	2	2.5	1.2	2.08	5.9	-.-	-.-	-.-	-.-
94-96	--	0	0	2.4	2.1	1.14	1.2	2.4	2.1	1.14	1.2
94-96	BTS	2	1	-.-	-.-	-.-	-.-	7.4	4.0	1.85	2.8
94-96	KYW	2	1	1.6	1.4	1.14	0.5	4.7	3.2	1.47	7.7

[1]Scion Age Codes: 0 = nongrafted; 1 = seedling; 2 = older juvenile cripple.
[2]Graft Type Codes: 0 = nongrafted; 1 = side; 2 = splice.
[3]Missing values indicate rootstock or scion were either removed or died.