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1) Screening the USDA soybean germplasm collection for Sp_1 variants.¹

Orf and Hymowitz (1976) using polyacrylamide gel electrophoresis revealed that the seed protein band called "A" by Larsen and Caldwell (1968) occurs at Rf 0.36 and the seed protein band called "B" occurs at Rf 0.42 (Rf = mobility relative to a bromophenol blue dye front in a 10% polyacrylamide gel anodic system using a pH 8.3 Tris-glycine buffer). The inheritance of these proteins (although the proteins were not characterized) was reported as being controlled by two codominant alleles at a single locus (Larsen and Caldwell, 1968). Orf and Hymowitz (1976) proposed the gene symbols Sp_1^a and Sp_1^b for the electrophoretic forms that occur at Rf 0.36 and Rf 0.42, respectively.

The genus <u>Glycine</u> Willd. is composed of two subgenera <u>Glycine</u> and <u>Soja</u> (Moench) F. J. Herm. (Hymowitz and Newell, 1979). The subgenus <u>Glycine</u> comprises the soybean, <u>Glycine</u> max (L.) Merr., and its closest wild relative <u>G. soja</u> Sieb. and Zucc. <u>Glycine</u> gracilis Skvortz. has been described as a species morphologically intermediate between <u>G. max</u> and <u>G. soja</u> (Skvortzow, 1927), but Hermann (1962) placed it under synonymy with <u>G. max</u>. For this report, Glycine gracilis has been separated from <u>G. max</u>.

The summary of the screening data is presented in Table 1; of the 2940 <u>Glycine max</u> accessions tested, 2617 accessions, or 89%, had the Sp_1^b allele. In the Asia collection, the remainder category is composed of soybeans introduced into the U.S. from Afghanistan, Burma, Indonesia, Malaysia, Nepal, Pakistan, Philippines, Taiwan, Thailand, the U.S.S.R. and Vietnam. Sources by Maturity Group (00 to VIII) for the Sp_1^b allele within the Named Variety Collection are 'Flambeau' (00), 'Grant' (0), 'Anoka' (I), 'Wells' (II), 'Cloud' (III), 'Clark' (IV), 'Hill' (V), 'Davis' (VI), 'Bragg' (VII) and 'Coker Hampton 266' (VIII). Sources by Maturity Group (00 to VI) for the Sp_1^a allele within the Named Variety Collection are 'Acme' (00), 'Evans' (0), 'Steele' (I), 'Amsoy' (II), 'Chestnut' (III), 'Bonus' (IV), 'Dixie' (V) and 'Rose Non-Pop' (VI).

Of the 359 <u>Glycine</u> <u>soja</u> accessions tested, 228 accessions, or 63.5%, had the Sp₁^b allele. The <u>Glycine</u> <u>soja</u> collection is made up of introductions from China, Japan, Korea, Taiwan and the U.S.S.R. All of the 39 <u>Glycine</u> gracilis accessions tested had the Sp₁^b allele.

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| Collection | S <u>p</u> 1 ^a | Spib | Mixture | Total |
|-------------------|---------------------------|------|---------|-------|
| Asia | | | | |
| Japan | 26 | 451 | | 477 |
| Korea | 83 | 334 | | 417 |
| China | 78 | 725 | | 803 |
| India | 52 | 167 | | 219 |
| Remainder | 8 | 156 | | 164 |
| Europe | 35 | 399 | | 434 |
| Other | | | | |
| Named Varieties | 36 | 296 | | 332 |
| Type Collection** | 10 | 83 | 1 | 94 |
| Glycine soja** | 131 | 223 | 5 | 359 |
| Glycine gracilis | | 39 | - | 39 |
| Totals | 459 | 2873 | 6 | 3338 |

*Data taken in part from Orf, 1976, 1979; Skorupska and Hymowitz, 1977.
**Type Collection 230 (T230) and five accessions of <u>Glycine soja</u> (PI 378,693B, PI 407,075, PI 407,080, PI 407,116 and PI 407,169) were mixtures containing both Sp1^a and Sp1^b seed.

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Distribution of Sp_1 variants in the USDA soybean germplasm collection*

Table 1

Skortzow, B. V. 1927. The soybean-wild and cultivated in Eastern Asia. Manchurian Res. Soc. Publ. Ser. A. Nat. Hist. Sec. No. 22: 1-8.

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2) Soybean linkage test between Ti and Le seed proteins.

The F_2 linkage results between the <u>Ti</u> locus and <u>Le</u> locus are presented in Table 1. In the table a = <u>Ti</u> <u>Le</u>, b = <u>Ti</u> <u>le</u>, c = <u>ti</u> <u>Le</u> and d = <u>ti</u> <u>le</u>. The parents used in the cross were in repulsion phase. Percentage recombination was obtained from the ratio of products following Immer and Henderson (1943).

The <u>Ti</u> and <u>Le</u> genotypes were determined using previously described procedures (Orf and Hymowitz, 1979; Orf <u>et al.</u>, 1978). The <u>Ti</u> gene controls the Kunitz trypsin inhibitor and the <u>Le</u> gene controls a seed lectin. The results indicate these two genes are not linked.

Table 1

Soybean F₂ linkage test of <u>Ti</u> and <u>Le</u> from the cross PI 196,168 (ti Le) x 'Norredo' (Ti^a le)

| a | b | С | d | Sum | %R |
|----|----|----|---|-----|----|
| 59 | 17 | 15 | 5 | 96 | I |

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3) Variation in percent seed oil in related nodulating and non-nodulating F_2 plants and F_3 progenies from three soybean crosses.

The soybean (<u>Glycine max</u> [L.] Merrill) uses both combined nitrogen from the soil and symbiotically fixed nitrogen from the air if effective nodules are present on its roots. Both sources of nitrogen are required for maximum yields at reasonable costs. In the absence of nodules, yields can be brought up to the level of those where effective nodules are present, but only with a high rate of nitrogen fertilizer application. Where both systems of nitrogen utilization are operating an increase in available nitrogen in the soil usually is accompanied by a reduced activity of the nitrogen fixing bacteria (<u>Rhizobium japonicum</u>). Such behavior would seem to result in a sort of buffer action that should reduce plant-to-plant variation in nitrogen utilization as expressed by seed yield and protein percent.

Liu and Hadley (1976) reported in several crosses that phenotypic variances for seed protein percent among non-nodulating $(rj_1 rj_1)$ F₂ plants averaged 1.6 times those of their nodulating (Rj_1) sibs. Estimates of environmental variance components, however, were made from very small samples of the homozygous non-nodulating parent, noduleless (CO) 'Clark' and the normal nodulating P₂ parents. Normal Clark is (CN). Heritability estimates, therefore, were not as accurate as desirable. A similar study was needed which included more adequate estimates of environmental components of variance from CO and CN or similar lines to be applied to populations of segregating generations.

It would seem appropriate for estimates of variance from the CO parental line to be subtracted from the phenotypic variance of F_2 , $\underline{rj_1}$ $\underline{rj_1}$ plants to estimate the genetic component of that F_2 sub-population. Similarly estimates of variance obtained from plants of CN could be subtracted from the phenotypic variance of F_2 , $\underline{Rj_1}$ $\underline{Rj_1}$ plants to estimate the genetic variance component of that F_2 sub-population. In a like manner, variances among hills of CO plants and hills of CN plants could be used as estimates of the non-genetic components of phenotypic variances among F_3 hills of $\underline{rj_1}$ $\underline{rj_1}$ and $\underline{Rj_1}$ $\underline{Rj_1}$ sub-populations respectively.

This report presents seed oil data from parental lines, P_1 (which is CO in our case) and P_2 , the normal counterpart of CO (which is CN), and F_2 and F_3 hybrid generations associated with three soybean crosses. Percent oil was chosen because it can be measured easily and in small seed quantities by nuclear magnetic resonance (NMR). Furthermore, the correlation between percent oil and percent protein is negative but quite high.

All crosses had CO as the female parent. One had 'Mandell', one had 'Wisconsin Black' and one had a Genetic Type Collection line, T245, as the male parent. Mandell has about 19% oil whereas Wisconsin Black and T245 have about 16%.

Plants of parental lines and F_2 's were grown approximately 30 cm apart in rows approximately 38 cm apart and 5.8 m long. Rows of CO, CN, P_2 and F_2 plants were randomized in blocks, one block for each cross. Seeds were harvested by individual plant and dried to 4% moisture. Oil percentages were estimated by NMR. Sixteen seeds from each plant were inoculated and grown in a mixture of sand and vermiculite for six weeks after which they were examined for the presence of nodules. If all seedlings had nodules their parental F_2 plant was assigned the genotype $Rj_1 Rj_1$, if none had nodules the F_2 parent was $rj_1 rj_1$ and if some had nodules while others did not the F_2 parent plant was classified as $Rj_1 rj_1$. Variances in oil percentages among CO, CN, P_2 and F_2 plants involved in three soybean crosses (1976)

Table 1

| Cross $(P_1 \times P_2)$ | CO (P ₁) | CN | P ₂ | $\underline{Rj_1Rj_1}(F_2)$ | $\underline{rj_1rj_1}$ (F ₂) |
|-----------------------------|----------------------|-----------|----------------|-----------------------------|--|
| C <mark>O x Ma</mark> ndell | 3.85 (40) | 1.81 (39) | 2.04 (37) | 2.56 (47) | 3.15 (62) |
| CO x T245 | 3.86 (21) | 1.80 (20) | 5.77 (18) | 3.18 (59) | 5.26 (55) |
| CO x Wisconsin Black | 2.77 (40) | 1.65 (40) | 9.62 (35) | 3.65 (83) | 4.36 (64) |

*Number in sample in parentheses.

Table 2

Variances in seed oil percentages among progeny hills of CO_1 , CN, P_2 and F_3 's involved in three soybean crosses (1977)

| Cross $(P_1 \times P_2)$ | CO (P ₁) | CN | P ₂ | $\underline{Rj_1Rj_1}(F_3)^*$ | <u>rj</u> rj (F ₃ |
|---|----------------------|----------|----------------|-------------------------------------|-------------------------------------|
| CO x Mandell CO x T245 CO x Wisconsin Black | 0.58(14) | 0.18(14) | 1.10(14) | 1.07 (92) 0.76 (87) 1.10 (92) | 1.58 (92) 0.99 (86) 1.22 (87) |

*Degrees of freedom in parentheses, pooled over two replications. In some cases there were missing hills in one or both replications.

Table 3

Heritability estimates (broad sense) for variation in seed oil percentages among F_2 plants and F_3 progenies associated with three soybean crosses

| | F ₂ plant | s (1976) | F ₃ progen | ies (1977) |
|---|-------------------------------------|---------------------------------|-------------------------------------|-------------------------------------|
| Cross | <u>Rj₁Rj₁</u> | rj ₁ rj ₁ | <u>Rj₁Rj₁</u> | <u>rj₁rj₁</u> |
| CO x Mandell CO x T245 CO x Wisconsin Black | 0.29 0.43 0.55 | -0.22 0.27 0.36 | 0.80 0.76 0.87 | 0.64 0.41 0.29 |

Sample sizes ranged from 20 to 40 within crosses but totaled 99 for CN. Those for CO ranged from 21 to 40 and totaled 101 over all three crosses. Data from these samples were applied to F_2 material. Estimates for the F_3 material came from eight hills of CO plants (16 over two replications) and eight hills of CN (16 over two replications).

 F_3 progenies consisted of two hills per F_2 family. One hill each of these, plus eight hills of each parental line as well as CN, were planted in each of two replications. Ten seeds were planted per hill. Entries were completely randomized within each replication. Hills were planted 30 cm apart in rows 76 cm apart. Each hill was harvested separately and its seed were sampled for NMR analysis.

Variances in seed oil percentages were higher for $F_2 \underline{rj_1} \underline{rj_1}$ plants than for their $F_2 \underline{Rj_1} \underline{Rj_1}$ sibs in all three crosses (Table 1) although significantly so only in the cross CO x T245. But variances of CO plants were significantly larger than those of CN plants. As a result the estimated environmental variance components for $\underline{rj_1} \underline{rj_1} F_2$ plants were greater than those for $\underline{Rj_1} \underline{Rj_1} F_2$ plants and estimates of heritabilities (in the broad sense) were lower for the noduleless than for the nodulated F_2 sub-populations (Table 3).

Evidence for genetic variance in the noduleless sub-population, in fact, is questionable because the variances among $\underline{rj_1}$ $\underline{rj_1}$ segregates (Table 1) were not significantly greater than that of the CO parent. Significant components for genetic variances, however, were present in the $\underline{Rj_1}$ $\underline{Rj_1}$ F₂ sub-populations in two of the crosses (CO x T245 and CO x Wisconsin Black).

Variances of $F_3 \underline{rj_1} \underline{rj_1}$ progeny hills were larger than those of F_3 <u>Rj_1</u> <u>Rj_1</u> progeny hills but significantly so only in cross CO x Mandell (Table 2). Variances of CO hills, however, were significantly greater than those of CN hills. Therefore the environmental component in the F_3 progeny variances should be larger in the $\underline{rj_1} \underline{rj_1}$ than in the $\underline{Rj_1} \underline{Rj_1}$ subgroup. Variances of $F_3 \underline{Rj_1} \underline{Rj_1}$ progenies were significantly larger than those of the CN parent indicating a real genetic variance component in the former in each of the three crosses. Only one variance of $F_3 \underline{rj_1} \underline{rj_1}$ progenies, however, was larger than that of the CO parent, in the cross CO x Mandell. Apparently no genetic component existed in the $F_3 \underline{rj_1} \underline{rj_1}$ progenies of the other two crosses. Estimates of heritabilities of differences among F_3 progeny hills are lower for the $rj_1 \underline{rj_1}$ portion of the population (Table 3).

We used only the noduleless Clark parent (CO) of the crosses or its normal counterpart (CN) for estimating environmental variance components. We did not use the P₂ parent because in each case it was normal $(Rj_1 Rj_1)$ for nodulation and would seem inappropriate for use with the noduleless $rj_1 rj_1$ portions of the segregating generations. We did not even use the P₂ parents for estimates to apply to the $Rj_1 Rj_1$ portions because such use would make comparisons between $rj_1 rj_1$ and $Rj_1 Rj_1$ subgroups unfair. If the P₂'s had been used in crosses CO x T245 and CO x Wisconsin Black estimates of h² for F₂'s would have been quite low because variances of T245 and Wisconsin Black plants were surprisingly high.

The data presented here certainly do not suggest that selection in the noduleless portion of F_2 or F_3 would be more effective than selecting in the nodulated portion. In fact they suggest the opposite. A significant genetic variance component in the $\underline{Rj}_1 \ \underline{Rj}_1 F_2$ sub-population indicates there are genetic differences for utilizing combined nitrogen from the soil, fixed

nitrogen from the air or both. The sub-population made up of related $\underline{rj_1} \ \underline{rj_1}$ plants should have the same array of genotypes except for genes on the chromosome segment that carries the $\underline{rj_1}$ locus. A failure of this portion of the population to express a significant genetic variance suggests that in our material genetic variation exists for the system involved with fixed nitrogen but cannot express itself in the absence of Rj_1.

Reference

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Relay cropping of soybeans and oats.

One possibility of increasing land productivity in Illinois is to double crop soybeans following wheat. This practice has been limited to the southern half of the state because of the shorter growing season in the northern half. A modification of double cropping known as relay cropping might allow the earlier establishment of soybeans in wheat or oats and extend the northern limit of double cropping in the state. Considerable work has been reported concerning double cropping, but relatively little has been published regarding relay cropping with soybeans (Brown and Graffis, 1976; Lassiter, 1973).

We have begun a study to determine the responses of 14 soybean cultivars representing Maturity Groups I through IV to relay planting in oats. We hope this study will help to answer the question, "Does the soybean breeder have to look for genotypes that differ from those of current cultivars adapted to monoculture in order to exploit efficiently the relay cropping environmental situation?"

<u>Materials and methods</u>: 'Lang' oats were planted April 14, 1978 in rows 41 cm apart. The unusually late planting was forced upon us by continual rains and the late arrival of spring. All the soybean cultivars (see Tables 1 and 2) were planted on May 27, 1978. The experimental design was a split plot with three replications. Monoculture and interplanting (relay planting) were the main plots and were arranged as randomized complete blocks. Subplots (cultivars) consisted of four rows 3.4 meters long and 41 cm apart. A space of 82 cm was left between adjacent plots.

On July 19, the oats were harvested by combine set to cut a height of 51 cm to obtain a maximum yield of oat grain with a minimum amount of removal of soybean plant tissue. Data were taken from the soybeans for lodging, plant height, and number of branches per plant just prior to harvest. Yield was estimated by harvesting 3 m of the two middle rows of each plot. The beans were harvested as they matured between September 19 and October 16.

Results and discussion: Tables 1 and 2 contain data for the traits measured on the soybean cultivars in relay cropping and in monoculture, respectively. Final values were calculated to determine significant differences and

| Cultivar | Yield (kg/ha) | Lodging | Height (cm) | Branches/plant |
|------------|---------------|---------|-------------|----------------|
| Wells | 618 a | 1.2 | 49 | .14 |
| Corsoy | 803 ab | 3.0 | 52 | .21 |
| Harcor | 918 ab | 3.0 | 56 | .58 |
| Hark | 1,067 abc | 2.3 | 55 | .48 |
| Amsoy 71 | 1,221 cd | 2.7 | 58 | .46 |
| Beeson | 1,431 cde | 2.6 | 62 | .88 |
| Elf | 1,445 cde | 3.0 | 45 | .43 |
| Wayne | 1,517 de | 3.1 | 72 | 1.78 |
| Cumberland | 1,560 de | 2.5 | 62 | 1.30 |
| Woodworth | 1,645 def | 1.9 | 67 | 1.75 |
| Union | 1,720 ef | 3.1 | 70 | 2.01 |
| 0akland | 1,745 ef | 2.9 | 70 | 1.66 |
| Cutler 71 | 1,845 ef | 3.7 | 80 | 2.07 |
| Williams | 2,076 f | 2.3 | 68 | 1.88 |
| Χ | 1,403 | | | |

Values for plant traits of 14 soybean cultivars (relay cropped in oats)

Table 1

Table 2

Values for plant traits of 14 soybean cultivars (monoculture)

| Cultivar | Yield (kg/ha) | Lodging | Height (cm) | Branches/plant |
|------------|---------------|---------|-------------|----------------|
| Hark | 2,452 a | 3.1 | 88 | 1.22 |
| Amsoy 71 | 2,764 ab | 3.1 | 105 | 2.17 |
| Corsoy | 2,788 ab | 3.1 | 105 | 1.85 |
| Cumberland | 2,953 abc | 3.3 | 91 | 2.70 |
| Woodworth | 3,116 abc | 3.3 | 98 | 2.32 |
| Elf | 3,284 abc | 1.1 | 58 | 2.51 |
| Beeson | 3,317 abc | 3.2 | 101 | 2.13 |
| Williams | 3,328 abc | 3.1 | 104 | 2.08 |
| Cutler 71 | 3,441 bcd | 3.0 | 129 | 1.89 |
| Oakland | 3,735 bcd | 2.1 | 104 | 3.36 |
| Harcor | 3,772 cd | 3.1 | 109 | 2.09 |
| Wells | 3,794 cd | 3.1 | 105 | 1.30 |
| Wayne | 3,835 cd | 3.1 | 129 | 1.71 |
| Union | 4,372 d | 3.0 | 121 | 1.20 |
| X | 3,359 | | | |

those yields not followed by the same letter are considered significantly different. An analysis of variance showed significant effects of cultivars. The interaction indicates that the cultivars respond differently when grown in different cultural systems. This is very important because if such an interaction holds over more environments it would indicate the need to evaluate cultivars in a relay cropping system before recommendations should be made about which cultivar to use in such a system. Also it would indicate that the breeder must select for performance under these conditions.

The results of the correlations made are given in Table 3. Correlations of special interest are the r values of yield in oats vs. yield in monoculture, and height vs. yield in oats. The low or perhaps non-existent correlation of yield (in oats) vs. yield (monoculture) was expected because of the previously mentioned interaction of cropping systems and soybean cultivars. The relatively high correlation between height and yield within the relay cropping system probably results from those with later maturity being able to take greater advantage of the remaining growing season after oat harvest, i.e., grow more after oat harvest and thus yield more. There are several possible explanations for the poor yields of several varieties. One was that some of the plots had poor germination because of dry conditions at Urbana that lasted from about April 20 to June 15. Most of the lower yielders were the earlier cultivars that were setting pods at the time of oat harvest. Regrowth from these was minimal. They may have yielded more had they been planted later, at about the time of heading of the oats. Some of the better yielders indicate, however, that a very real potential exists for relay cropping to increase land productivity.

The oat yields (from the relay cropping treatment) averaged about 2,000 kg/ha. If bean yields of 2,000 kg/ha are added to such oat yields the system of relay cropping could be viewed as being profitable--especially if these levels of yields can be proven to be reliable. Further study will be conducted in 1979 with a similar experimental design and at several locations in the state of Illinois.

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Correlations of various plant traits of soybeans

r = .2138 N.S. Yield (monoculture) Yield (in oats) VS. Lodging (in oats) r = -.2372 N.S. VS. Lodging (monoculture) Height (monoculture) r = .7184 * *Height (in oats) VS. Branches/plant (monoculture) r = .1564 N.S.Branches/plant (in oats) VS. Lodging (in oats) r = .2245 N.S.Yield (in oats) VS. r=.8856** Yield (in oats) Height (in oats) VS. r = .6600*Branches/plant (in oats) vs. Yield (in oats)

*Significant at 5% level.
**Significant at 1% level.
N.S. = not significant.

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1) Induced cytoplasmic sterility in soybeans.

One of the M_4 progenies of PK-71-39 soybean irradiated with 10 Kr gamma rays showed segregation for sterility in soybean in 1976. It had 18 sterile plants and 4 normal plants, indicating that a single dominant gene was responsible for sterility. The sterile plants had no seeds and, therefore, this appeared to be a dead end for this mutant. Nevertheless, the 4 normal plants were separately harvested and their progenies evaluated in 1977. The results were very interesting, as indicated in Table 1.

| | No. of | plants |
|-------------|---------|---------|
| Progeny no. | Sterile | Fertile |
| 1 | 35 | 2 |
| 2 | 53 | 1 |
| 3 | 5 | 0 |
| 4 | 22 | 1 |
| Total | 115 | 4 |

Breeding behavior of normal plants from segregating rows

Table 1

As evident from the table, all the 4 progenies consisted primarily of sterile plants with occasional fertile ones. Pooled over all progenies, there were 115 sterile plants and 4 fertile plants. The progenies of these 4 normal plants were again evaluated in 1978. The results were very similar to what was observed in 1977, as indicated in Table 2.