UPOV
International Union for the Protection of New Varieties of Plants

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THE UNITED STATES MOLECULAR MARKER WORKING GROUP: BACKGROUND FOR THE USE OF DNA MARKERS IN DUS

Document prepared by experts from the Seed Association of the Americas (SAA)

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SOYBEAN CULTIVATION AND IMPROVEMENT

1. Soybean (*Glycine max* (L.) Merr.) was introduced into North America as a forage crop in the 18th century (Hymowitz and Haran, 1983). Primary usage as forage continued until the late 1930s after which there was a rapid transition to harvesting seed for oil and meal. Soybean breeders began programs of genetic improvement by developing segregating populations through controlled hybridizations between Plant Introductions.

DEVELOPMENT AND USE OF MOLECULAR MARKERS IN SOYBEAN

2. Molecular markers have a long history of use in soybean which reflects the evolution of these technologies (Figure 1). Application of molecular marker technology spans numerous disciplines within the plant sciences (Figure 2).

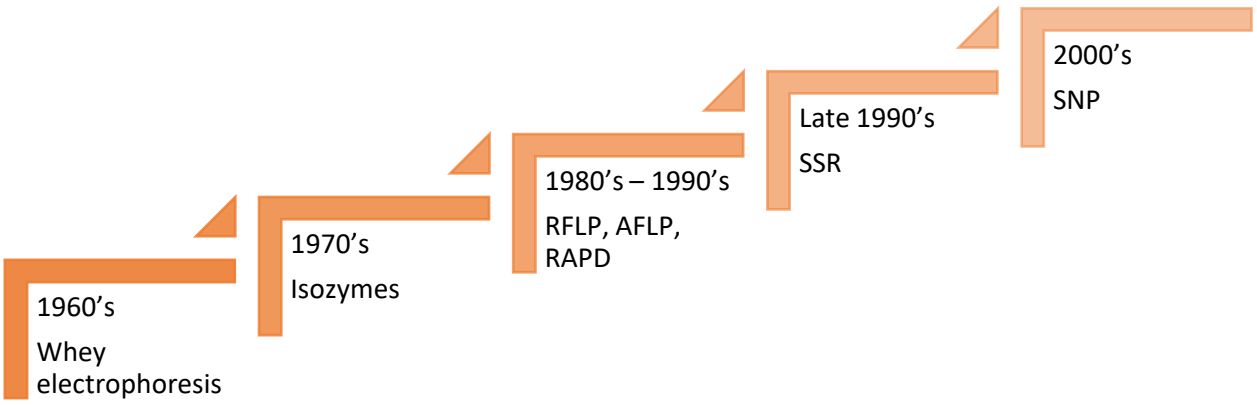

Figure 1 Evolution of molecular marker technologies in soybean.

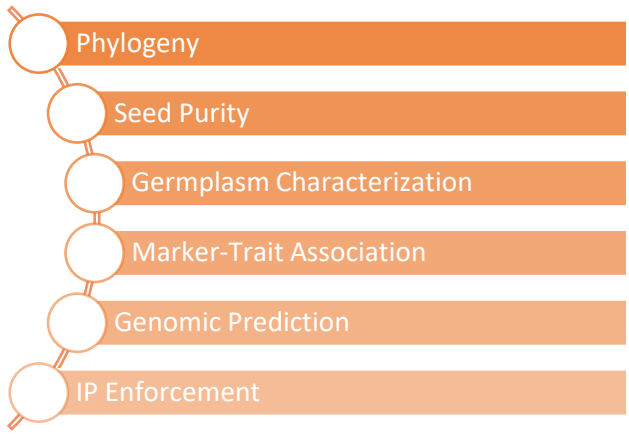

Figure 2 Applications of marker technology in plant sciences.

UPOV AND THE NEED FOR INTELLECTUAL PROPERTY

3. The International Union for the Protection of New Varieties of Plants (UPOV) is an inter-governmental organization whose mission is to provide and promote an effective system of plant variety protection, with the aim of encouraging the development of new varieties of plants, for the benefit of society. The UPOV Convention provides a *sui generis* form of intellectual property protection which has been specifically adapted for the process of plant breeding and has been developed with the aim of encouraging breeders to develop new varieties of plants.

4. Morphological and physiological data constitute the foundation upon which candidate varieties are evaluated against DUS requirements – that the variety is distinct (D) from all other variety whose existence is a matter of common knowledge, it must also be uniform (U), and be stable (S).

5. The development of a new soybean variety takes from 3-6 years depending upon the strategies employed. If breeders wish to recoup investments, they must obtain intellectual property (IP) rights on new varieties they have developed. A robust IP system enables incentives for continued investment in crop improvement.

APPROVED USES OF MOLECULAR MARKER DATA

6. UPOV accepts the use of marker data to help determine distinctness either when there is verification of the reliability of the link between the marker and the characteristic, or when a combination of phenotypic differences and molecular distances can be used to identify those varieties which need to be compared with candidate varieties (document TGP/15/1).

7. UPOV includes isozyme characteristics in an annex to the Test Guidelines for DUS testing of soybean candidate varieties (document TG/80/6). The annex states that “The following Annex contains a list of characteristics derived by using electrophoresis and a description of the method to be used. UPOV decided to place these characteristics in an Annex to the Test Guidelines, thereby creating a special category of characteristic, because the majority of the UPOV member States is of the view that it is not possible to establish distinctness solely on the basis of a difference found in a characteristic derived by using electrophoresis. Such characteristics should therefore only be used as a complement to other differences in morphological or physiological characteristics. UPOV reconfirms that these characteristics are considered useful but that they might not be sufficient on their own to establish distinctness. They should not be used as a routine characteristic but at the request or with the agreement of the applicant of the candidate variety.”

8. Experts from France proposed the use of “other tools for the identification of a variety” as “supporting evidence” when morphological differences, as determined by experts, are small but not sufficient alone to determine distinctness. See flax example in Table 1, where differences exist, but none sufficient for establishing distinctness (document TWA/28/17).

9. The International Seed Federation (ISF) supports the use of molecular markers to measure genotypic conformity as a means to helping determine Essentially Derived Varieties (EDV) status (ISF 2012). The Naktuinbouw similarly concluded that "genetic conformity can be used as a tool to predict essential derivation" (Teunissen 2013).

Table 1 Differences observed between [flax] variety A and variety B in DUS trials in France and Belgium (1992 and 1993) (UPOV 1999 TWA/28/17)

N° UPOV TG/57/6	F-1992	F-1993	B-1992	B-1993	Remark
1) Plant: natural height	- 2,6 cm	- 1,8 cm	+ 9,4 cm	- 1,3 cm	Minimum distance (5 cm) achieved once and inconsistency of differences.
2) Stem: length	+ 1,0 cm	+ 2,2 cm	+ 4,9 cm	+ 0,5 cm	Minimum distance (5 cm) never achieved but consistency of small differences.
3) Flower: size of corolla	0	0	0	0	No difference
4) Sepal: dotting	+2	+1	+4	+2	Characteristic highly influenced by environment. Minimum distance of 4 achieved once and consistency of small differences.
5) Petal: color of crown	0	0	0	0	No difference
6) Petal: color of corola	0	0	0	0	No difference
7) Petal: longitudinal folding	0	0	0	0	No difference
8) Stamen: color of distal part	0	0	0	0	No difference
9) Anther: color	0	0	0	0	No difference
10) Style: color	0	0	0	0	No difference
11) Boll: size	+2	+1	+1	+1	Minimum difference (2) achieved once and consistency of small differences.
12) Boll: ciliation of false septa	0	0	0	0	No difference
13) Seed: weight per 1000 seeds	+ 0,2 g	+ 0,4 g	+ 0,6 g	+ 0,4 g	Minimum distance (0,5 g) achieved once and consistency of small differences.
14) Seed: color	0	0	0	0	No difference
15) Time of beginning of flowering	- 1 day	+ 1 day	+ 2 days	+ 1 day	Minimum distance (3 days) never achevived and inconsistency of differences.

RECOGNIZING POTENTIAL PITFALLS

10. Use of relatively few SNPs has been shown to be effective in variety identification (Yoon et al.,2007). However, for DUS chief pitfalls that have been foreseen in the use of molecular marker data are:

i) A potential reduction in the level of IP currently provided through PVP by reducing evidence for distinction to one or a few base pair polymorphisms.
ii) A stringent level of uniformity requiring the entire genome to be uniform across individual plants of a variety.
iii) Increase in costs of breeding, seed increase, and purity assessment with a disproportionate burden falling upon smaller and medium-sized breeding organizations.

11. These concerns can be addressed by using a sufficiently large set of SNPs and by examining SNP distances between and within varieties and so establishing thresholds for distinctness and uniformity that reflect current levels of SNP diversity for genotypes which have already been granted varietal status based on morphologically expressed characteristics.

INTER AND INTRA-VARIETY HETEROGENEITY CONSIDERATIONS

12. Plant genomes, even of inbred organisms, harbor residual variability because selection has been demonstrated within homogeneous genetic pools:

i) Genetic variation underlying the expression of agronomic traits was observed within F5–derived lines (Byth and Weber, 1968).
ii) Maize inbred lines of doubled haploid derivation were found to accumulate variation in agronomic traits via mutation (Sprague et al. 1960; Russell et al. 1963).

iii) Continued response to selection was observed in maize after more than 90 generations (Dudley and Lambert, 2004).

iv) Residual diversity for agronomic traits was found within each of the soybean varieties “Benning”, “Haskell”, and “Cook” (Fasoula and Boerma, 2007)

v) Residual SNP variation has been found in the soybean variety “Williams 82” though most of the genome is fixed. (Haun et al. 2011).

vi) Rasmusson and Phillips (1997) reported generation of de novo variation in elite germplasm pools.

vii) McClintock (1984) suggested that environmental stress may be a trigger of genomic change allowing for continued adaptation under selection.

viii) Due to heterosis in soybean, selections can retain heterozygosity in genomic regions under selection (Fasoula and Boerma 2007)

13. Thus, to determine SNP thresholds for distinctness using large (>1000) SNP sets, intracultivar genetic heterogeneity must be addressed. This is necessary because characteristics used to determine distinctness must be sufficiently uniform to prevent derivation of a new and distinct variety solely by re-selection of residual diversity within a protected variety.

GENETIC BASE OF CULTIVATED SOYBEAN IS NARROW

14. The genetic base of US soybean is acknowledged to be quite narrow due to bottlenecking effects that continued post-domestication reducing both the number of original founder landraces. As few as 35 ancestral founders account for 95% of the genetic variation in soybean (Gizlice et al., 1994). However, sufficient genetic diversity exists to allow the improvement of U.S. soybean production through the creation and select of new genotypes (Figure 3). Specht et al. (2014) estimate that genetic improvement contributes between 67% to 85% of soybean yield gains on U.S. farms in the span from 1924 – 2010. In the future, breeders will have more challenges to face due to demands for more production in a more challenging and unpredictable environment.

15. Diwan and Cregan (1997) concluded that “most commercial soybean cultivars arise from hybridization between members of an elite group of genotypes, and the amount of genetic variability among these cultivars is small.” Thus, new cultivars are often indistinguishable based on these standard pigmentation and morphological traits.

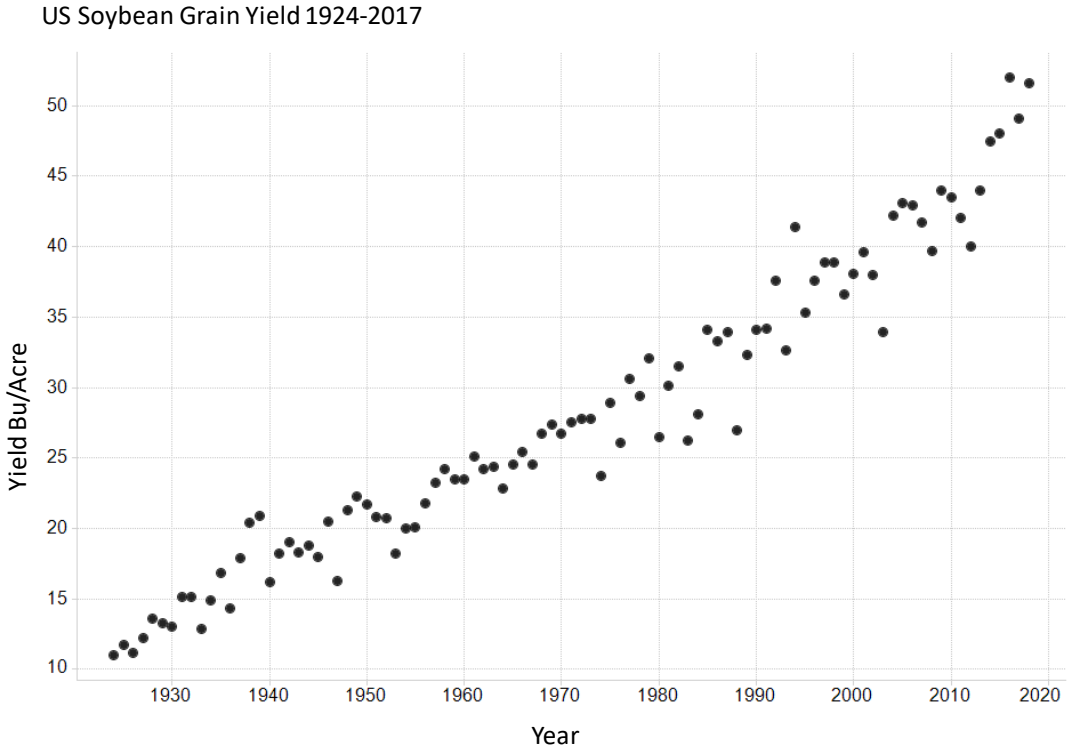

Figure 3 United States soybean grain yield 1994-2017, in bushels per acre.

CURRENT CHALLENGES IN USING MORPHOLOGICAL AND PHYSIOLOGICAL CHARACTERISTICS FOR DUS IN SOYBEAN

16. For soybean, UPOV (1998 TG/80/6) lists 20 morphologically expressed characteristics comparisons of which provide information upon which determinations of DUS are made. There are clearly many challenges associated with field-based DUS trials which are very labor-intensive and expensive. Genotypic by environmental effects are difficult to model and unanticipated environmental effects introduce error which cannot be explained with even the best models.

17. Additional challenges arise from the expression and distribution of morphological characteristics. For example, not all possible characteristic states are found in equal frequency, thereby reducing overall discrimination power (Law et al., 2011; Kumar et al., 2017). In addition, correlations among morphological characteristics further limits the number of available different character states overall (Law et al., 2011).

18. Reference collections are already large (Song et al., 1999) numbering >4000 in the U.S. system (Sukhapinda, 2016) and > 1000 in Brazil (Ribeiro et al., 2103), and the rate of increase of accessions in reference collections can reach several hundred per annum (UPOV, 2005).

19. Clear test criteria and statistically-grounded methods are prescribed for conducting and evaluating DUS trials. Experience of knowledgeable experts is also given consideration in determining levels of distinctness and in establishing reliability of differences. In all cases thresholds must be established whereby a final judgment of distinctness is made.

TWO STUDIES AND THEIR OBJECTIVES

20. The United States Molecular Marker Working Group has initiated two lab-based studies to aid in determining SNP marker-based distinctness thresholds for Soybean:

1) Development and validation of methods to characterize soybean varieties using SNPs. Here we report that bulk sampling using 10-20 plants and the use of 6k SNPs or subsets thereof were reliable and appropriate methodologies for determining genetic distances in soybean. Levels of SNP heterogeneity, and both intra- and inter-laboratory repeatability were measured, which must factor into distance thresholds for determining DUS and essential derivation.
2) SNP profile, pedigree, morphological, and physiological distance examination of soybean varieties. We have selected a set of historically important U.S. soybean varieties. All varieties have either been granted DUS on the basis of morphological and/or physiological characteristics or they are included in the U.S. PVP Office reference set. Taking into account levels of residual heterogeneity, intra- and inter laboratory SNP profiling error, and based upon alignments of SNP, morphological, physiological and pedigree comparisons among DUS varieties we will propose i) SNP marker based thresholds of similarity between soybean varieties that could be used to help determine Distinctness, Uniformity, and Stability and ii) thresholds which could be used to help determine eligibility as an Essentially Derived Variety.

REFERENCES

Byth D. E., Weber C. R. 1968. Effects of genetic heterogeneity within two soybean populations. I. Variability within environments and stability across environments. Crop Sci 8: 44–47.

Diwan N., and Cregan P. B. 1997. Automated sizing of fluorescent-labeled simple sequence repeat (SSR) markers to assay genetic variation in soybean. Theor Appl Genet 95:723-733.

Dudley, J. W. and R. J. Lambert. 2004. 100 generations of selection for oil and protein in corn. Plant Breeding Reviews 24:(part1)79-110.

Fasoula V. A., Boerma H.R. 2007. Intra-cultivar variation for seed weight and other agronomic traits within three elite soybean cultivars, Crop Sci 47:367-373.

Gizlice Z., Carter T.E., Burton J. 1994. Genetic Base for North American Public Soybean Cultivars Released between 1947 and 1988. Crop Science. 34. 1143.

Haun W. J., Hyten D. L., Xu W. W., Gerhardt D. J., Albert T. J., Richmond T., Jeddeloh J. A., Jia G., Springer N. M., Vance C. P., Stupar R. M. 2011. The composition and origins of genomic variation among individuals of the soybean reference cultivar williams 82. Plant Physiol 155:645-655.

Hymowitz, T. and J. R. Harlan. 1983. Introduction of soybeans to North America by Samuel Bowen in 1765. Econ. Bot. 37: 371-379.

ISF, 2012. ISF View on Intellectual Property Adopted in Rio de Janeiro, Brazil, 28 June 2012 32pp Nyon, Switzerland.

McClintock, B. 1984. The significance of responses of the genome to challenge. Science 226:792-801.

Rasmusson, D. C., and R. L. Phillips. 1997. Plant breeding progress and genetic diversity from de novo variation and elevated epistasis. Crop Sci. 37:303–310.

Sprague G. F., Russell W. A. and Penny, L. H. 1960. Mutations affecting quantitative traits in selfed progeny of doubled monopploid maize stocks. Genetics 45: 855-865.

Sprague G. F., Russell W. A. and Penny, L. H. 1963. Mutations affecting quantitative characters in long-time inbred lines of maize. Crop Sci. 3: 175-178.

Suhre J. J., Weidenbenner N. H., Rowntree S. C., Wilson E. W., Naeve S. L., Conley S. P., Casteel S. N., Diers B. W., Esker P. D., Specht J. E., Davis V. M. 2014. Soybean Yield Partitioning Changes Revealed by Genetic Gain and Seeding Rate Interactions. Agronomy Journal. 106. 1631.

Teunissen, H. 2013. A technical perspective on essentially derived varieties. UPOV seminar on EDVs Oct 22, Geneva.

Yoon M. S., Song Q. J., Choi I. Y., Specht J. E., Hyten D. L., Cregan P. B. 2007. BARCSoySNP23: a panel of 23 selected SNPs for soybean cultivar identification. Theor Appl Genet. 114(5):885–99.

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