

The Harvest Index as Breeding Criteria in Soybean [*Glycine max* (L.) Merrill]

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Summary

Crosses of soybean genotypes (cv. Atlanta, MG 0; cv. Romantica, MG 00; cv. Izidor, MG I and cv. Houta, MG I) with different meaning of the trait harvest index (HI) were made (2017) and studied (2018-2020) in the environmental conditions of the region of Central North Bulgaria, the town of Pavlikeni (43°24' N, 25°32' E, 144 m a.s.l.). The objectives of the study were: i) establishment of genotype variance by HI and the traits determined for it in the group of the parent genotypes and in their hybrid populations (F₂-F₃ generations); ii) assessment of the potential of different parent genotypes and development of the strategy for crosses for the improvement of the HI; iii) assessment of the capacity of the HI as main or additional selection criteria in the breeding programs for seed productivity of soybean. In F₂ and F₃ generation of each hybrid combination biometrical and variance analyses were conducted for the following traits: total yield of aboveground plant biomass (including seeds, stems and branches, without leaves -BMY, g); seed yield per plant (SY, g) and harvest index (HI). The correlation (r) and regression (b) between F₂ and F₃ were assessed on the base of the middle values in F₃ generation of each hybrid combination and its corresponding individual values per transgressive forms in HI. Based on the results obtained, the application of father component with high meaning of the HI resulted in breeding progress for seed productivity. It is reasonable to use the application of the harvest index as a breeding criterion in progenies of the crosses with father genotype as a donor with high value of the trait aboveground biomass. By phenotypic assessment of the HI as additional selection criteria recombinant lines with increased seed productivity were selected.

Key words

Glycine max (L.) Merrill, harvest index, breeding criterion, genetic improvement

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Introduction

Soybean is a crop with great economic importance for the human population because of the high protein and oil content (Butnariu and Butu, 2015). Soybean production increases worldwide every year. In relation to this, more attention could be spent on modern and adequate strategies for the increase of the plant productivity (Blažinkov et al., 2015; Popović et al., 2015; Popović et al., 2020).

The trait harvest index (HI) is a ratio of two complex characteristics, grain yield and total yield from the aboveground biomass. The analysis of HI is one of the main topics in plant biology, including plant physiology, genetics and breeding (Wnuk et al., 2013). The HI describes the plant capacity to allocate biomass (assimilates) into the formed reproductive parts, which is a marker for reproductive efficiency (Porker et al., 2020). It is an important trait for cereal crops, where the considerable progress in breeding aimed at higher yield is achieved mainly through selection for the harvest index (Mazid et al., 2013).

In the soybean crop the harvest index is traced more rarely, because of the interaction between the trait with maturity or seeding date. Another reason for the less attention to this trait is the fact that the harvest index in soybean measured at maturity does not include leaf and petiole dry weights. However, the results indicate that harvest index is a larger contributor to the progress of soybean yield improvements than biomass in China (Cui and Yu, 2005). Investigations on seed yield quantitative trait loci (QTL) QTL show that in soybean crop the increase of seed yield (SY) could be obtained by evaluation of biological yield or by improvement of the harvest index. According to the study of Todeschini et al. (2019) the soybean breeding progress in Brazil, one of the most important soybean producers worldwide, is a result of the efforts imputed to the increase of the harvest index. In many papers it has been demonstrated that HI is a stable cultivar trait for the soybean crop (Rosenzweig Rosenveig et al., 2016; Jain et al., 2017; Kawasaki et al., 2018). According to Spaeth et al. (1984) main factors like sowing density, long-lasting photoperiod, duration of the drought have no influence on the HI of the determinate and indeterminate soybean cultivars. In Europe the soybean cultivars have different periods of vegetative and reproductive phases, growth type and habitus, seed mass and other morphological characteristics (Naydenova and Georgieva, 2019).

There are no studies to explain how these differences in combination with the different growth technologies influence the harvest index. The information for the phenotype manifestation of the trait HI of the Bulgarian soybean cultivars under different environmental and production conditions is also scarce. In our previous studies on the cultivars Avigeya and Richi (MG I) a tendency for increase of the trait under conditions of early sowing and high sowing density was found. The same results concerning only early matured cultivars (MG 00 and MG 0) were published by Edwards and Purcell (2005). Possibilities for the improvement of HI as a result of the yield potential of the soybean crop by combinative breeding are presented in Bisht et al. (2018). The authors found that HI was under control of additive gene action by studying variability of the trait in early hybrid generations and noted that the HI could respond positively to the selection.

According to Rosenzweig et al. (2016) the efficiency of the selection by trait HI is stable and it does not depend on the season conditions and competition ability of the genotypes included in the hybrid populations. It was found that selection by trait HI in small plots or in individual plants in the selection nursery was more effective for the improvement of the seed productivity than the selection by seed yield (Trunova and Kochegura, 2016).

The present investigation aims are:

- Establishment of genotype variance by harvest index and the traits determined for it in the group of the parent genotypes and in their hybrid populations (F_2 - F_3 generations).
- Assessment of the potential of different parent genotypes and development of the strategy for crosses for the improvement of the HI.
- Assessment of the capacity of the HI as main or additional selection criteria in the breeding programs for seed productivity.

Material and Methods

Plant Material

In the investigation, crosses of the Ukrainian early matured cultivar Atlanta (MG 0) were used. According to the preliminary tests this cultivar possesses a moderate degree of manifestation of the harvest index (HI). The cultivar Atlanta is used as a mother plant, crossed with cultivars Romantica, MG 00; Izidor, MG I and Houta, MG I as father genotypes with high, low and middle level of HI respectively.

Field Experiment and Biometrical Measurements

The crosses were conducted in 2017 in the region of Pavlikeni (43°24' N, 25°32' E, 144 m a.s.l.), which is in the temperate continental climate zone with a well-established continental rainfall regime - with a maximum in May-June and a minimum in August-September.

In the period 2019-2020 second and third hybrid populations of the crosses were grown in the breeding nursery and monitored. The hybrid lines and the parent genotypes were sown in rows, with a distance of 70 cm between the rows and 10 cm inside the rows. A randomized block method was used.

Biometrical analyses were conducted at phase R8 - full maturity, after leaf fall (Purcell et al., 2014), of the following traits: total yield of aboveground plant biomass (including seeds, stems and branches, without leaves - BMY, g); seed yield/seed mass per plant (SY, g) and harvest index (HI, %). Twenty individual plants were selected and analyzed for calculation of middle arithmetic (\bar{x}), standard deviation (SD), scope of variability (Range), degree and frequency of transgressive forms in F_2 generation of each hybrid combination.

The transgression is presented according to the level of the trait increase in the hybrid combination in comparison with the mother parent. The F_2 hybrid generations, which are the transgressive forms of the harvest index, falling into the early groups of maturity (MG 00 and MG 0) were monitored in F_3 generation. Each progeny was sown in individual row 4m in length and 10 individual plants were used for the biometric analysis.

Statistical Analyses

According to the data from the parent genotypes for the period 2018-2020, by applying two-way analysis of variance including factors G = genotype and Y = year, the variance components of the investigated traits were calculated. The multiple comparison of the average values through the smallest statistically proven differences (LSD test at $P < 0.05$) was conducted. To assess the factorial influences, the main indicator η^2 (%) was used and it was calculated as relative share of the factorial to the total variance of the trait. The significance of genotypic differences by the traits HI and SY per plant between the generations of each cross was evaluated in F_3 generation by one-way analysis of variance.

The correlation (r) and regression (b) between F_2 and F_3 were assessed on the base of the middle values in F_3 generation of each hybrid combination and its corresponding individual values per plant /transgressive forms in HI/. The efficiency of the harvest index as an alternative breeding criterion for seed yield per plant was estimated.

Results

Phenotypical performance concerning the breeding trait harvest index and the characteristics defining this trait are presented in Table 1. On the base of these results the strategy for breeding was selected. It was found that the genotypic variance of the parents genotypes was significant for the traits total yield of the aboveground biomass per plant (BMY) $P < 0.05$ and harvest index (HI) ($P < 0.001$). The genotypic differences by the trait seed yield per plant (SY) were insignificant ($\eta^2_G = 5.3\%$, $P > 0.05$). The variance of the BMY was not affected by the specific reaction of the parents genotypes to the annual condition and the values of the main indicator η^2_{G*Y} for evaluation of this factorial influence were very low - 97% ($P > 0.05$). The results obtained for BMY of cultivar Isidor were superior in comparison with other father genotypes used.

Strong effect by the genotypic factor in combination with the annual conditions or separately on the performance of the trait HI ($\eta^2_G = 41.8\%$; $\eta^2_{G*Y} = 18.9\%$) was determined and the highest average values were detected for the cultivar Romantica (HI=47.3%) and the lowest were detected for the cultivar Isidor (HI=34.1%), respectively. The cultivar Atlanta, which was used as a mother plant in all crosses falls in homogenic groups with the father genotypes by the trait BMY, but it is significantly different by HI in comparison with the cultivars Romantica and Isidor.

In the second hybrid generation the families of all crosses showed almost identical average values for the trait HI and the traits depending it, SY and BMY (Table 2). On the other hand, the variance of the traced traits was affected by the father genotype. In F_2 population of the crosses with father genotype Romantica, the highest hybrid variability of the trait BMY per plant was found, while in the crosses with cultivar Houta as a father genotype, the trait SY and HI showed the highest level of variability.

Table 1. Mean values, significance of differences, analysis of variance and degree of factorial influences (η^2 %) for seed yield (SY), above-ground biomass yield (BMY) and harvest index (HI) of soybean cultivars used in the crosses

	♀Atlanta (MG 0)			♂Romantica (MG 00)			♂Isidor (MG I)			♂Houta (MG I)			LSD $P < 0.05$			G			Y			G*Y		
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	η^2	MS	η^2	MS	η^2	MS	η^2	MS	η^2
BMY (g)	58.8 ^{ab}	24.9	42.2 ^b	51.3 ^b	25.9	47.3 ^a	74.5 ^a	26.1	34.1 ^c	47.6 ^b	19.4	40.8 ^b	20.2	8.7	3.8	16.0%	4202**	15.7%	861ms	16.0%	4202**	15.7%	861ms	9.7%
SY (g)	24.9	42.2 ^b	47.3 ^a	25.9	47.3 ^a	40.8 ^b	26.1	34.1 ^c	34.1 ^c	19.4	40.8 ^b	8.7	20.2	3.8	5.3%	1374***	23.8%	389*	5.3%	1374***	23.8%	389*	20.2%	
HI (%)	42.2 ^b	47.3 ^a	34.1 ^c	47.3 ^a	34.1 ^c	34.1 ^c	34.1 ^c	34.1 ^c	34.1 ^c	40.8 ^b	29.4***	41.8%	270***	12.8%	18.9%	294***	12.8%	133***	41.8%	270***	12.8%	133***	18.9%	

Note: Values followed by same letters does not differ significantly at $P < 0.05$; ns – not significant, * - $P < 0.05$; ** - $P < 0.01$; *** - $P < 0.001$

Table 2. Performance of the monitored traits in F_2 hybrid population of the crosses

	BMY (g)						SY (g)						HI (%)											
	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD	Mean	Range	SD
♀Atlanta × ♂Romantica	37.9	18.0-90.4	17.4	16.1	7.5-37.9	7.0	16.1	7.5-37.9	7.0	1.0-82.0	20.0	42.9	35.3-48.8	3.7	42.9	35.3-48.8	3.7	42.9	35.3-48.8	3.7	42.9	35.3-48.8	3.7	50.0
♀Atlanta × ♂Isidor	37.9	22.9-64.4	11.7	16.3	10.1-27.7	5.7	16.3	10.1-27.7	5.7	23.0-33.0	21.0	42.6	34.6-50.7	4.2	42.6	34.6-50.7	4.2	42.6	34.6-50.7	4.2	42.6	34.6-50.7	4.2	60.0
♀Atlanta × ♂Houta	38.0	12.4-76.4	16.2	16.5	6.7-36.8	7.4	16.5	6.7-36.8	7.4	3.0-77.0	27.0	43.9	28.0-54.0	5.8	43.9	28.0-54.0	5.8	43.9	28.0-54.0	5.8	43.9	28.0-54.0	5.8	68.0

Positive transgressive forms by the trait HI were obtained in all cross combinations. In the crosses with the father genotype Houta, where both parents showed moderate levels of the monitored selection criteria, the level and frequency of the positive transgressions were highest. These results suggest a high specific combined capacity of the Houta variety. In the crosses with the cultivar Romantica, which possesses the highest harvest index, a higher rate of transgressions was observed, but with a lower frequency compared to those of the crosses with the cultivar Isidor, which possesses a low manifestation of the trait.

After the selection of the transgressive forms by the HI (HI > 42.4%), F₃ populations of the crosses differ more clearly in the mean values for the observed traits (Fig. 1A-C). The results show that in the cross where the father genotype possesses higher HI value - ♀Atlanta × ♂Romantica the achieved average results are the highest. The selection by harvest index demonstrates more significant parallel increase of the yield of biomass and seed yield in comparison with both parent components. It is important to note that the genotype variance in the F₃ population of this cross combination is significant for the SY per plant ($P < 0.05$) and for HI ($P < 0.001$), Table 3. It could be concluded that by using cultivar Romantica as a father parent, a useful breeding genotypic segregation in early generation (F₃) is achieved. Strong positive correlation between the SY and HI has also been found ($r_{HI F_3/SY F_3} = 0.96$), Table 4. This result allows the application of HI trait as selection criterion for seed yield elevation. The results obtained for the productivity trait confirm the efficiency of HI as alternative selective criterion (Table 3).

Table 3. Significance of genotypic variance in F₃ generation of family

	♀Atlanta × ♂Romantica	♀Atlanta × ♂Isidor	♀Atlanta × ♂Houta
BMV (g)	$P > 0.05$	$P > 0.05$	$P < 0.1$
SY (g)	$P < 0.05$	$P > 0.05$	$P > 0.05$

Table 4. Correlative /r/ and regressive /b/ coefficients of the observed traits in F₂ and F₃ generations

Hybrid combinations	♀Atlanta × ♂Romantica	♀Atlanta × ♂Isidor	♀Atlanta × ♂Houta
$r_{SY F_3/ HI F_2}$	0.37	0.18	0.71
$b_{SY F_3/ HI F_2}$	0.51	0.24	0.59
$r_{SY F_3/SY F_2}$	-0.74	0.59	0.40
$b_{SY F_3/SY F_2}$	-0.45	0.64	0.11
$r_{HI F_3/SY F_3}$	0.96	0.14	-0.44
$b_{HI F_3/SY F_3}$	0.48	0.25	-0.49

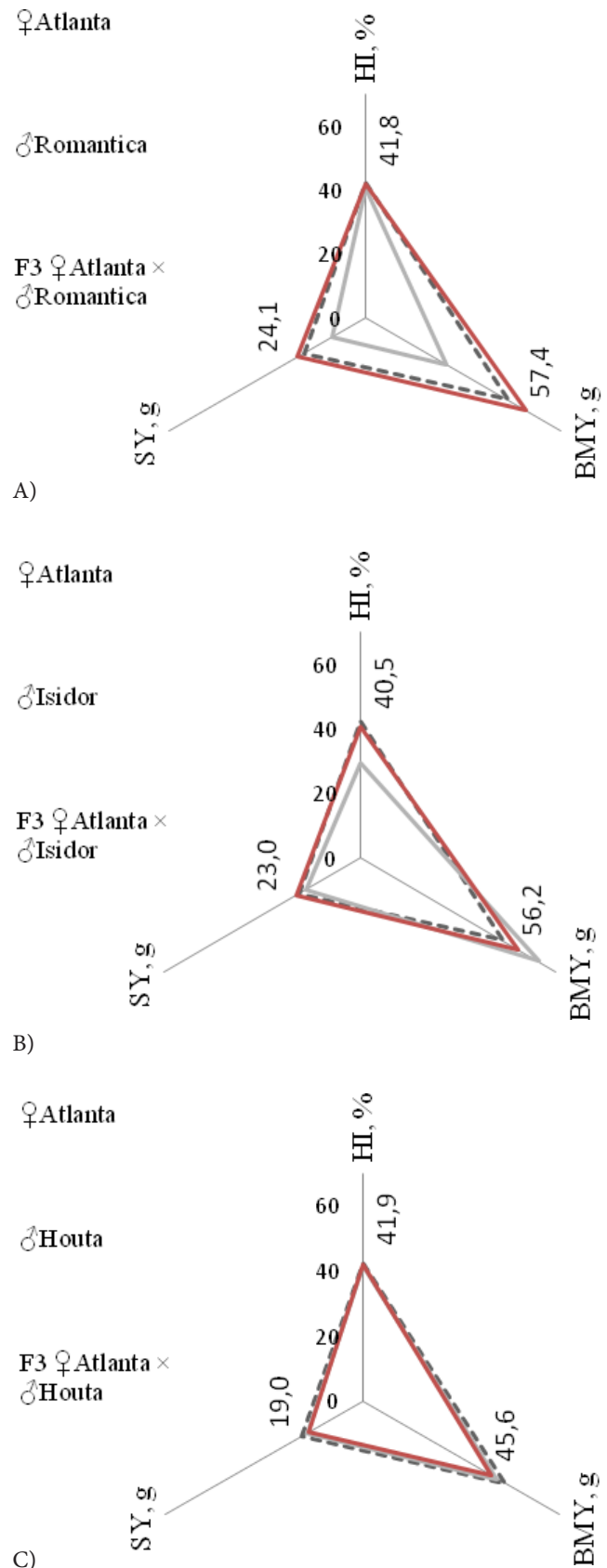


Figure 1 A-C. Performance of F₃ populations of the crosses by mean values for the observed traits

Discussion

Harvest index is a trait combining both parameters, seed yield and total yield from the aboveground biomass, and was studied as a potential for selective criteria in early generations of hybrid populations.

There were medium strength positive correlation (0.18–0.71) and regression (0.24–0.59) between HI of the selected recombinant F_2 lines and the average yield of seeds per plant for the respective F_3 generations. It could be concluded that the crosses, where the father genotype is a donor of the HI trait, the hybrid combination ♀**Atlanta** × ♂**Romantica** is the most effective for the breeding progress for seed yield by harvest index selection. Similar results were demonstrated by Reynolds et al. (2017) in wheat. After different strategies for crossing, the authors conclude that the complementary crossing between genotypes with high biomass and genotypes with high HI are described as a promising strategy to achieve genetic gains.

The environmental factors are important for the harvest index and especially the seasonal feature of the water supply and extreme temperatures during the reproductive development (Porker et al., 2020). The sustainable soybean yield under the growth conditions without irrigation in Bulgaria could be overcome with the use of early genotypes and the breeding program directed for development of early maturity cultivars (Aleksieva, 2015). The high value of the observed traits of the discussed hybrid combination in F_3 could be connected also with the early maturity of the parent genotypes, with the selection of very early and early hybrid plants, where the reproductive phases take place under conditions of better moisture supply.

In the cross combination ♀**Atlanta** × ♂**Isidor**, where the mother genotype possesses high value of the selection trait, the achieved average results have been close to the cross discussed above in relation to the traits BMY and SY per plant. The values of HI were almost at the same level as in the mother genotype. In this cross the selection on the base of phenotypically expressed individual productivity in F_2 was more effective in comparison to the selection by HI (Table 3). At the same time the significant genotypic variance in the progenies from F_3 generation was found only for the harvest index trait. In this case it was reasonable to use HI as additional criterion for selection. The results obtained coincide with understanding that the seed yield potential is largely a consequence of increased biomass production. By the results published in the paper of Cui et al. (2008) the SY increase could be genetically explained by either biological yield enhancement and/or harvest index improvement.

There is tendency in the modern plant breeding to direct the breeding process in the way where not just HI is in the focus, but also both traits, HI and biomass or even biomass itself (Aisawi et al., 2015).

The cultivar **Isidor** used as father genotype in the described cross combination is the standard cultivar for the country. In Bulgarian breeding programs the number of branches is monitored because of the application of large rows technology for soybean growing. As a result, the yield from the branches in Bulgarian standard cultivars has a high relative contribution to the yield per plant. On the other side, the selection for the increased number

of branches is the indirect selection for the elevated yield of total aboveground biomass. From this point of view and based on the results presented, it is reasonable to include the harvest index as selective criterion for early generations in the improvement of selection programs of soybean crop.

In the cross combination with the highest variability in F_2 - ♀**Atlanta** × ♂**Houta** there has been found selection progress for the HI in comparison with the parent forms. This result is in accordance with the one established by Bisht et al. (2018) data, where the trait HI in soybean is under adaptable gene control and it is responsive to phenotypic selection. At the same time F_3 population of this particular cross shows the lowest average values for the both traits, aboveground biomass yield and seed yield per plant. It has been demonstrated that the selection only by HI results in elevation of the trait meaning with low effect on the total dry matter production (Flohr et al., 2018a).

According to the values of the correlative ($r_{SY F_3/ HI F_2} = 0.71$) and regressive coefficient ($b_{SY F_3/ HI F_2} = 0.59$) presented in Table 4, the efficiency of the selection by HI is significantly higher in comparison with the selection by individual productivity. However, in F_3 generation the seed yield per plant and HI were negatively connected. This result makes reasonable the thesis that improvement in the yield will need selection for increased biomass production with parallel monitoring of the HI (Mitchell and Sheehy, 2018). The obtained hybrid material from the cross combination ♀**Atlanta** × ♂**Houta** demonstrates the possibility for such tandem selection because it has been observed that there is genotypic variability in the trait yield of aboveground biomass in F_3 generation.

Conclusion

Based on the presented results, the trait hybrid index could be suggested as main and additional selected criteria in the early progenies of the cross combinations.

The application of father component with high meaning of the harvest index trait has resulted in breeding progress for seed productivity.

It is reasonable to apply the harvest index as a breeding criterion in progenies of the crosses with father genotype as a donor with high value of the trait aboveground biomass.

By phenotypic assessment of the HI as additional selection criterion, recombinant lines with increased seed productivity were selected. In 2021 they were tested and stabilized in the F_4 generation. The best lines will be selected in F_5 - F_6 generation of the cross combinations.

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