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Selectable Traits for Yield Improvement in Double Crop Soybeans at High Plant Density with Late Planting

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Abstract: The late planting of double crop soybean (*Glycine max* L.) at high plant density affects plant growth during both vegetative and reproductive stages resulting in a loss of seed yield. The objectives were to examine genetic and growth variation at high plant density and to determine and combine selectable growth traits into a selection index for the development of primary breeding criteria. Five soybean genotypes, Camp, Eunha, HS 287, Iksannamul and Pureun were used in field experiments conducted over three years. Yield was significantly ($p<0.05$) dependent upon number of pods per plant and number of pods on primary stem. Number of pods on primary stem was significantly ($p<0.05$) and negatively correlated with internode length and branch angle, but positively correlated with harvest index. Highly weighted variables under the first principal component which explained 49.2% of the total variation included internode length, lodging and number of pods on primary stem. The second principal component explained an additional 23.5% of the total variation. These two components explained 72.7% of the total variation in all the variables. This study accompanied with the multiple regression analysis ($R^2 = 0.88$) with four key variables (number of pods on primary stem, internode length, branch angle and lodging) indicates that these growth traits are selectable agronomic components in a series of breeding programs to improve ultimate seed yield effectively at high plant density condition along with a short growing season.

Key words: Early planting · Plant density · Internode · Primary stem · Selection index · Soybean

INTRODUCTION

Soybeans (*Glycine max* L.) have been planted as a double crop after the harvest of small grain crops such as winter wheat and barley in many regions [1]. In the double crop soybean, growers frequently employ high plant density rather than standard plant density per unit area to minimize yield loss due to late planting. Late planting is intentional in double cropping after winter wheat or barley and should be finished between June 1 and June 30 in regions (e.g. Midwest and South Central states, Southeastern Asia) for best yield [2]. Late planting of soybeans as a double crop at high plant density causes a change in plant development and morphology and restricts a full vegetative growth often to the benefit of reproductive growth, resulting in a loss of seed yield [3, 4]. Soybean growers in the regions are often recommended to plant full-season varieties as early in April as possible or to finish planting by May 15 to increase potential yield by taking full advantage of a growing season.

Our previous studies demonstrated significant yield loss (up to 22%), particularly when number of pods was reduced [4, 5] and lodging occurred during the R4-R6 stages [6, 7]. Lodging was significantly associated with wider stem diameter [8-10]. Other studies suggested that environmental factors influencing growth characteristics such as stem growth and primary stem diameter were light quantity and quality due to high plant density [11-13]. Soybean cultivars have been developed in response to specific demands on growth traits in a variety of growth environments. A concern in many investigations [14, 15] has been the development of cultivars and their related cultural practices optimized under high plant density. However, few studies have focused on the development of soybean cultivars with better growth characteristics adapted to high plant density and late planting.

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Objective choice of selection index with differential weight of each trait by breeders is difficult. Selection index maximizes genetic gains in a desired direction and generally estimates the value of an individual for an aggregate genetic constituent [16, 17]. Index selection is one of methods in which useful target traits are selected simultaneously and has been applied to develop better cultivars with target traits [18]. Despite of the usefulness of selection index and breeding value as the value of an individual, in some cases it is not easy to determine definite breeding objectives of certain traits precisely. But, as far as breeding values of the target traits are recognized, the traits should be incorporated into index selection breeding program in corporation with other selection methods.

Principal component analysis (PCA) has been used to discover the dimensionality of a data set and to identify new meaningful underlying variables under particular growth environments [19-21]. PCA has applied to examine genotype main effect in grain crops, including *Hordeum vulgare* L. [21] and *Glycine* accessions (*G. max*, *G. soja,* etc.) [20]. In a study with azuki bean [19], the genetic relationships among the bean populations collected from different areas were determined by PCA, in which the first principal component (PC 1) explained 20.0% of the total variation. PC 1 was the combination of variables that explains the greatest amount of variation.

We were interested in effective selection criteria in our long-term breeding programs to develop new cultivars adapted to high plant density and a relatively short growing season. The objectives of the study were to examine growth responses of soybean genotypes to high plant density during a short growing season and to determine selectable growth traits for the ultimate development of higher yield cultivars which are well adapted to a high plant density and late planting.

MATERIALS AND METHODS

Plant Materials: A total of five soybean genotypes (Camp, Eunha, HS 287, Iksannamul, Pureun) were used. Camp and HS 287 were from collections maintained by the breeding program at the National Honam Agricultural Research Institute and the remainders were cultivars developed by the National Institute of Crop Science, Korea.

A long primary stem genotype included Iksannamul and Pureun (76±4 to101±8 cm in plant height) and a short primary stem genotype included Camp and HS 287 (50±5 to 45±6 cm) (Table 1). Eunha was employed as an intermediate stem genotype (63±5 cm). Within the long primary stem, Iksannamul possessed lateral (2° axis) branches with 4.2±0.4, more than those of Pureun which had branches with 1.2±0.3. Short stem Camp and HS 287 were 4.1±0.4 and 2.3±0.3 in number of branches, respectively. Eunha had 3.2±0.04 in number of branches. Other growth characteristics, including maturity of genotypes are presented in Table 1.

Field Experiments: Field experiments were conducted in 2004, 2005 and 2006 at the National Honam Agricultural Research Institute Agronomy farm, Iksan (126.97° E, 36.00° N), Korea. Monthly mean temperatures during the growing season (June to October) in Agronomy farm ranged from 11 (October) to 30 (August) °C, while monthly mean precipitations ranged from 3.71 (October) to 24.29 (August) cm. August was the warmest month, with low mean temperature of 23 and high mean of 30°C. Each plot consisted of four rows spaced at 0.60 m apart and 4 m in length. Seeds were planted in fields on the 4th (2004), 2nd (2005) and 5th (2006) of June in a randomized complete block design with three replicates following a harvest of winter wheat or barley. Seedlings were thinned to achieve designated high plant density (i.e., 66 plants m^{-2}) within a row. Recommended 33 plants m^{-2} were considered as standard plant population density and were used as control in this study. Fertilizer was applied to test plots at 1.0-1.7-1.5 kg ha^{-1} (N-P-K) before sowing of seeds. No irrigation was provided for plants in field experiments. Border rows were planted to eliminate edge effects.

Table 1: Major vegetative and reproductive traits of genotypes with different stem growth habits and lateral branch types used at high plant density

Variety	Primary stem	Plant height (cm)	Inflorescence	R1 (d)	Maturity (d)	Branch[c] (no)
Camp	Short	50±5	DT[a]	2 Aug. (58)	10 Oct. (127)	4.1±0.4
Eunha	Intermediate	63±5	DT	31 Jul. (56)	9 Oct. (126)	3.2±0.4
HS 287	Short	45±6	DT	26 Jul. (51)	29 Sep. (116)	2.3±0.3
Iksannamul	Long	76±4	DT	4 Aug. (60)	9 Oct. (126)	4.2±0.4
Pureun	Long	101±8	IT[b]	13 Jul. (38)	27 Sep. (114)	1.2±0.3[a]

Determinate (monotelic) inflorescence; [b] Indeterminate (polytelic) inflorescence; [c] Includes primary branches except for subbranches

A minimum of three samples was randomly taken from each of the two central rows in each plot for growth traits, including number of mature pods per plant, seeds per pod, number of nodes, 100-seed weight and plant dry biomass. All plants from two rows were harvested for mean seed yield (kg ha^{-1}) and other important growth traits, including dry biomass. Leaf samples were collected from two young leaves, the second fully expended leaf and the third leaf, at the same reproductive stage (R1) [22] and leaf areas were measured on a LI-3100 electronic area meter (LI-COR, Lincoln, NE). Total dry biomass was determined by drying almost physiological mature plants (R7±2 d) in a dry oven for 3 d at 65±5°C, at which only few leaves and petioles dropped, but at least one normal pod on the main stem turned yellow. Pods at full maturity (R8), at which 95% of the pods reached their mature pod color, were separately collected from each plant.

Lodging was based on a visual rating from 1 to 9 as follows: 1, almost all plants erect and 9, all plants prostrate [23]. Harvest index, the ratio of seed mass to total plant biomass, was calculated as total dry seed weight divided by total aboveground dry biomass per plant [24]. Plant dry biomass was estimated by total weight of all harvested mass per plant.

Statistical Analysis: The data over three years were subjected to PROC CORR [25] to estimate correlation coefficients among examined growth characteristics. Principal component analysis (PCA) was used to determine major factors among significantly correlated growth characteristics in various stem growth genotypes under two plant densities [26]. The principal components were computed from the standardized variables. All standardized data were subject to PCA by PROC PRINCOMP and used to perform the regression of the dependent variables on the set of component variables by PROC REG [25].

RESULTS

Seed Yield and Pod Number on Primary Stems: Analysis of variance showed significant effects of genotype, plant density and environment on seed yield and morpho-physiological traits such as pod particularly on primary stem and lodging (Table 2). Interactions between genotypes and their growing environments (GxE) were significant on number of pods on primary stem and lodging, but were not significant on seed yield (Table 2). The mean seed yields of three genotypes, Camp, HS 287 and Iksannamul significantly ($p<0.05$) were increased at high plant density (66 plants m^{-2}) as compared to the yield at standard plant density (33 plants m^{-2}, Fig. 1). Number of pods on primary stem at high plant density significantly decreased in genotypes, Eunha, Iksannamul and Pureun, but increased in Camp and HS 287 relative to the standard plant density. Lodging was not significant in Eunha at high plant density as compared to the standard.

Correlation Coefficients among Growth Traits: Number of pods on primary stem was found to be significantly ($p<0.05$) negative correlation with lodging (-0.71**), but positive correlation with harvest index (0.60*) (Table 3). Yield was significantly ($p<0.05$) correlated with number of pods per plant (0.66*) and number of pods on primary stem (0.62*), but negatively correlated with internode length (-0.61*).

Principal Component Analysis and Multiple Regressions among Growth Traits: The first principal component explained 49.2% of the total variation and dominated by highly positive internode length (0.42) and lodging (0.41) (Table 4). The coefficients (eigenvectors) of the second principal component showed a positive relationship with some variables (e.g., number of pods) and received large

Table 2: Analysis of variance for selected reproductive and morpho-physiological traits including seed yield in soybean genotypes with different primary stem lengths grown in different plant population densities for three years

		Mean squares		
Source of variation	df	Yield	Pod on primary stem	Lodging
Genotype (G)	4	443,836.5**	18.6*	1.3**
Density (D)	1	34,569.0**	6.8*	1.0**
Environment (E)	5	3,540.0*	114.7*	4.7*
G x D	4	NS	97.6*	NS
G x E	20	NS	3.2**	1.9*

*, ** Significantly different at the 0.05 and 0.01 levels of probability, respectively. NS, Not significant at the 0.05 level of probability

Table 3: Correlation coefficients among principal growth traits for five soybean genotypes grown at high plant density in a short growing season across three years

Characteristics	Primary stem length	No branch	Branch length	Branch angle	Internode length	Petiole length	Leaf area index	No pod per plant	No pod on primary stem	Harvest index	Lodging
No branch	0.68*										
Branch length	0.33	0.48									
Branch angle	0.64*	0.54	0.29								
Internode length	0.46	0.27	0.20	0.62*							
Petiole length	0.69*	0.63*	0.26	0.24	0.24						
Leaf area index	0.26	0.49	-0.08	0.35	-0.21	0.37					
No pod per plant	0.23	0.46	-0.05	0.08	-0.51	0.44	0.75**				
No pod on primary stem	-0.44	-0.28	-0.28	-0.57*	-0.82**	0.17	0.20	-0.27			
Harvest index	-0.85**	-0.59*	-0.21	-0.47	-0.59*	-0.67*	-0.05	0.06	0.60*		
Lodging	0.70*	0.59*	0.53	0.58*	0.72**	0.41	-0.18	-0.20	-0.71**	-0.67*	
Yield	-0.30	0.02	-0.37	0.01	-0.61*	-0.24	0.51	0.66*	0.62*	0.55	-0.59*

*, ** Significant at the 0.05 and 0.01 levels of probability, respectively. N = 60

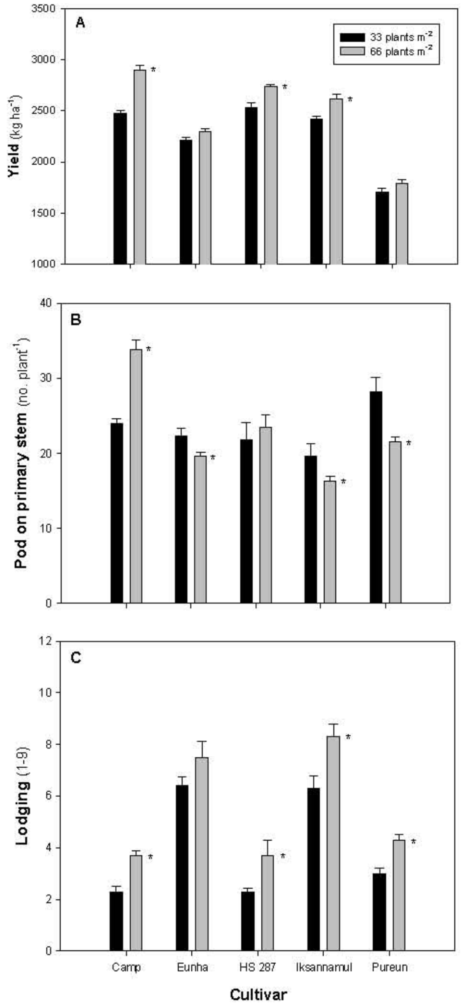

Fig. 1: Seed yield and selected morpho-physiological traits in soybean genotypes with different primary stem lengths and grown in two plant population densities (33 and 66 plants m^{-2}) for three years. * Significantly different at the 0.05 level of probability.

Table 4: Results of principal component analysis (PCA) among growth traits of soybean genotypes at high plant density across three years

	PCA			
Characteristics	1st	2nd	3rd	4th
Eigenvalue	4.43	2.12	0.98	0.68
Contribution (%)	49.20	23.50	10.90	7.50
Cumulative contribution (%)	49.20	72.70	83.60	91.20
Eigenvector				
Yield	- 0.38	0.28	0.18	0.38
Branch length	0.22	0.12	- 0.82	0.33
Branch angle	0.26	0.48	0.21	0.43
Internode length	0.42	0.06	0.30	0.11
Lodging	0.41	0.17	- 0.21	- 0.04
No of pod per plant	- 0.27	0.50	- 0.22	- 0.26
No pod on primary stem	- 0.43	- 0.02	- 0.24	- 0.16
Leaf area index	- 0.16	0.58	0.11	- 0.11
Harvest index	- 0.34	- 0.24	- 0.02	0.67

contributions from leaf area index (0.58), number of pods per plant (0.50) and branch angle (0.48) (Table 4). The second principal component explained an additional 23.5% of the total variation. In fact, the first two components explained 72.7% of the total variation in the nine-variable dataset. The first and second principal components showed relatively large variations (eigenvalues, 4.43 and 2.12, respectively in Table 4). These eigenvalues were greater than zero and represented exact linear dependency, but the rest had small or modest variances (eigenvalues less than 0.98).

Correlation coefficients of selected growth characteristics that largely contributed to the total variation in the first and second PCA are shown in Table 5. In the first principal component, correlation coefficients of internode length and lodging were 0.89** and 0.86**, respectively. Negatively significant correlation coefficients were found in number of pods on

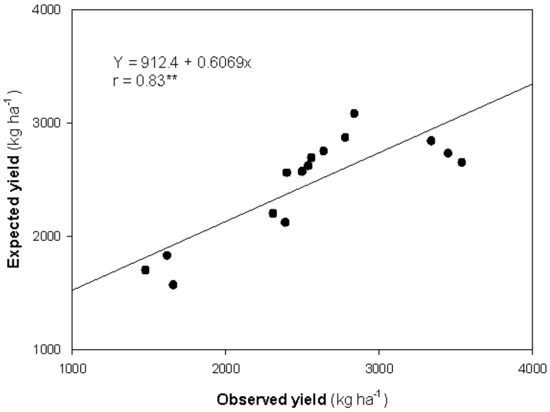

Fig. 2: Linear regression and correlation coefficient between expected yield and observed yield at high plant density (66 plants m^{-2}).

Table 5: Correlation coefficients of selected growth traits from the first and second principal component analysis (PCA)

	PCA	
Characteristics	Component 1	Component 2
Yield	-0.79**	0.40
Branch length	0.46	0.17
Branch angle	0.54	0.70*
Internode length	0.89**	0.08
Lodging	0.86**	0.25
No of pod per plant	-0.57	0.73**
No pod on primary stem	-0.90**	-0.04
Leaf area index	-0.34	0.84**
Harvest index	-0.71**	-0.35

*, ** Significantly different at the 0.05 and 0.01 levels of probability, respectively. N = 60

Table 6: Multiple linear regression analysis of soybean growth traits as principal components at high plant density across three years

Variable (X)	Multiple regression equation (Y)	R^2
Internode length (X_1)	$Y = 347.5 - 13.86X_1$	0.61*
Branch angle (X_2), X_1	$Y = 299.8 - 22.72X_1 + 2.77X_2$	0.79**
Lodging (X_3), X_2, X_1	$Y = 273.2 - 15.92X_1 + 3.26X_2 - 5.53X_3$	0.86**
Primary stem pod (X_4), X_3, X_2, X_1	$Y = 204.5 - 10.90X_1 + 3.33X_2 - 4.63X_3 + 1.36X_4$	0.88**

*, ** Significantly different at the 0.05 and 0.01 levels of probability, respectively. N = 60

primary stem (-0.90**), yield (-0.79**) and HI (-0.71**). In the secondary principal component, we found three positive correlation coefficients in leaf area index (0.84**), number of pods per plant (0.73**) and branch angle (0.70*) (Table 5). The four major growth characteristics which were primarily selected by PCA and correlation coefficients (refer to Table 4 and 5) were subjected to multiple regression analysis (Table 6). From the regression analysis, coefficients of determination (R^2) were 0.61 in internode length (X_1) alone, 0.79 in branch angle along with X_1 and 0.86 in lodging along with X_1 and X_2.

Figure 2 shows simple regression and correlation coefficient (r) between expected seed yield and observed one at high plant density (66 plants m^{-2}). Expected seed yield, which was estimated by the multiple regressions, was formulated by the previous seed yield data in standard plant density (33 plants m^{-2}). Correlation coefficient (r) was 0.83 ($p<0.01$) between expected value of seed yield and observed (harvested) value of seed yield at high plant density.

DISCUSSION

Growth traits of seed yield and number of pods on primary stem were significantly influenced by genotype, plant density and environment. Seed yield in genotypes increased more in dense plant populations than in the standard because of increased numbers of plants per unit area. Seed yield was significantly associated with number of pods on primary stem and internode length (Table 2) [27]. Variation in number of pods either per plant or on primary stem depended upon population density [27]. Internode length was significantly ($p<0.05$) and positively correlated with lodging, but negatively correlated with number of pods on primary stem and harvest index (HI). These significant associations suggest that seed yield was likely to be genetically or morphologically dependent upon number of pods on primary stem and that number of pods on primary stem decreased as internode length increased because of high plant density [28].

One of our interests in this study was to determine selectable traits that are used for the development of cultivars adapted to high plant density farming with a short growth period. We used eigenvectors of principal component analysis to define selection index (SI) criteria and also estimated the coefficient of determination (R^2) to determine the associations among interest traits. Estimated the portion of each selectable trait, which contributes to SI and breeding values, gives an estimate of the transmitting ability of the parent. Generally, the highly correlated variables (e.g., number of pods on primary stem, internode length, HI and lodging) clearly contributed to a large variation of growth traits shown by the first principal component analysis (Table 3). Almost half of the total variance received a significant contribution from the growth traits (e.g., number of pods on primary stem, internode length, etc.) and 23.5% of the total variance received from other variables (e.g., number of pods per plant and leaf area). In fact, a major portion (72.7%) of the total variation in populations was efficiently explained by these variables [29, 30].

Significant coefficients in both principal components imply that these variables (e.g., internode length, pods on primary stem and lodging) were primary growth traits that responded considerably to high plant density (Table 5). Also, other multiple regressions ($R^2 = 0.88^{**}$) demonstrate that four growth traits, internode length (X_1), branch angle (X_2), lodging (X_3) and number of pods on primary stem (X_4), were potential key factors which particularly responded to plant density (Table 6). These detectable variations caused by plant density were the result of genetic constitution in genotypes [15, 30], but genetic plasticity on these traits in various plant densities remains to be investigated in the future. However, SI criteria determined in this study help select outperformed the best individuals for breeding cycles [18].

Some growth traits (e.g., number of pods per plant and number of seeds per pod) have simultaneously bred, based on assigned differential weight values in breeding programs, including a single seed descent method [31]. The selected potential traits with higher index score can be used in further breeding progress to develop higher yield cultivars adapted to dense plant populations under the condition of a relatively short growth period. A breeding strategy can primarily be set to increase number of pods on primary stem, but retain or reduce internode length to minimize lodging problems in dense plant populations. We believe that the genetic variation of these traits in parental individuals is only gradually made available for breeding of the traits through breeding systems including the processes of directional selection and the breakup of undesirable linkage blocks [32, 33] by other means available. Based on the multiple selectable traits defined in this study, the key in breeding strategies is the flow of genetic variation in parental populations from the cryptic state to the transposable one for breeding efficiency within a given time.

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