Agricultural Science & Technology, 2010, 11(3): 87–89, 97

Screening Index for Low Phosphorus Tolerance at Seedling Stage

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Abstract **[Objective]** Simple and reliable identification criteria used in screening phosphorus efficiency of soybean at seedling stage was discussed. **[Method]** Using the high and low phosphorus soil pot experiment, the soybean seedling stage's 5 criteria including RPH, RAW, RRW, RAPC and RRPC were studied. **[Result]** Low phosphorus stress had the low effects on relative plant height, the coefficient of variation was only 9.07%, has not achieved the remarkable level with its its criteria's relevance. Low phosphorus stress had the higher effects on relatively above-ground dry weight and relative dry weight of root, relatively above-ground phosphorus concentration and relative root phosphorus concentration, its coefficient of variation was also big, in turn is 26.67%, 22.68%, 24.01% and 15.87%, The correlation coefficient among the indicators had significantly or extremely significantly positive correlation. **[Conclusion]** Relatively above-ground dry weight and relative dry weight of root, relatively above-ground phosphorus concentration can be used as an important indicator of comprehensive evaluation of screening phosphorus efficiency of soybean at seedling stage, while the relative root phosphorus concentration can be used as secondary screening index.

Key words Soybean; Low phosphorus stress; Screening criteria

Phosphorus is one of the essential macroelements in the plant growth and development, which participates directly in a variety of important physiological and biochemical processes of plants and plays an extremely important role in promoting plant growth and development and metabolism, as well as increasing production. However, most of the world agricultural soils are severely P-deficient[1]. In china, the area of soil phosphorus deficiency is 6.121×10^7 hm^2, in which there are 18 Provinces and autonomous regions of which the percentage of P-deficient area in the Province's total land area is more than 75%, and seriously hampering the local crop production and product quality. Relying on fertilizer inputs can alleviate this contradiction, but because of the special chemical behavior of phosphate, it is susceptible to be fixed by the Fe-Al oxides in acid soils and calcium carbonate compounds in calcareous soils, resulting in the phosphate utilization is not high, which leading to phosphorus deficiency problem remains serious. According to statistics, since the application of phosphate fertilizer has been carried out in China, the amount of chemical phosphate fertilizer that has been applied was accumulated up to 3.4×10^7 t, in which more than 1.5×10^7 t is fixed by soil, in particular, the fixation capacity of acidic red soil and lateritic soil in south of China to phosphorus is stronger, resulting in phosphate utilization is very low, which only accounts for 10% – 20%. The phosphate utilization of soybean is lower, due to the long-term application of phosphate fertilizer, the majority of soil has become the potential phosphorus pools[2]. In view of the phosphorus deficiency of majority of soil is "Genetics phosphorus deficiency"[3–5], therefore, to study the genetic and physiological and biochemical mechanisms of efficient absorption, transport and use of soil phosphorus nutrients of crops, as well as to use the genetic resources of phosphorus efficiency plants and improve the phosphorus efficiency crop breeding techniques has become an important key topic in the whole world[1].

From the economic and environmental considerations, a low-input and high-efficient way is needed to solve the problem of the P-deficient in crop production. A large number of studies have shown that plants of different species and different varieties (lines) have different capacities on the use of soil phosphorus, there are large differences on genotypes, in which the difference between different species are obvious[6–7], which provides genetic resources for the screening and breeding Phosphorus efficient genotypes, as well as to provide a good way for the improving of the utilization of phosphorus. In the same time, the establishment of simple and reliable screening evaluation index is extremely important for screening and breeding. Therefore, in this study, eight different soybean genotypes that selected from field experiments were used as materials, and low phosphorus soil pot experiment was conducted to evaluate the efficiency of its phosphorus, as well as to provide theoretical basis for the screening of phosphorus efficient soybean genotypes.

Materials and Methods

Experimental materials

Eight soybean genotypes [*Glycine max* (L.) Merrill] in Heilongjiang Province were taken as experimental materials, the names were D03, D05, D17, D18, D31, D34, D37 and D38 (purchased from Heilongjiang Academy of Agricultural Sciences and the Institute of Soybean of Northeast Agricultural University).

Experimental soil

The experimental soil was the farming soil purchased from Zhaodong City, Heilongjiang Province, it was calcareous chernozem, texture loam, and the collection depth of soil was 20 cm, and the previous crop was maize. Soil physical and

Received: February 20, 2010 Accepted: March 31, 2010
Supported by Foundation of Heilongjiang Provincial Department of Education "Biological Basis of the Roots from Different Soybean Genotypes Adapting to Low-phosphorus Soil" (10551033); Scientific Research Startup Foundation for Doctor Program of Mudanjiang Normal University (MSB200912).
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chemical properties were shown in Table 1, in which the available P content was low, while the total P content was high. The specific determination was according to "Soil Agricultural Chemistry Analysis Method"[8].

Table 1 Physical and chemical properties of tested soil

Total N// g/kg	Total P// g/kg	Slowly available K// g/kg	Alkalihydrolyzable N// mg/kg	Available P// mg/kg	Rapidly available K// mg/kg	Organic matter// g/kg	pH
1.60	0.43	748	133.9	5.9	112	32.0	8.2

Experimental materials

Experimental design Pot experiment was carried out indoors of Horticulture Station, Northeast Agricultural University on May 6, 2008. Two treatments was set up, respectively, which were as follows ① high phosphorus (CK), 3.03 g of superphosphate, 0.075 g of urea [$CO(NH_2)_2$], 0.075 g of potassium sulfate (K_2SO_4) per kg soil were applied. ④ low phosphorus, nitrogen, potassium and non-P fertilizer were applied, each treatment was repeated for four times. The air-dried soil that through the 3 mm sieve was fully mixed with P fertilizer and other nutrient elements, and then they were put into a plastic bucket (20 L), 10 seeds of soybean that was full and with the same size was sowing in each bucket. Emergence for 10 days later, after thinning, three plants that of the same size were left. After 32 d of cultivation, five indicators such as plant height, plant biomass of the aboveground parts, biomass of root, aboveground P content and P content of root was detected[8].

There were natural differences on biological traits between different Soybeans, in order to eliminate this difference, relative indicators were used to measure the capacity of tolerance to low phosphorus stress between different genotypes. Relative index = (measured value of low P treatment/measured value of high-P treatment (CK)) × 100%, including the relative plant height (RPH), relative aboveground dry weight (RAW), relative root dry weight (RRW), relative aboveground P concentration (RAPC), relative root P concentration (RRPC).

Data processing Excel 2003 and DPS 8.0 data analysis software were used for the statistical analysis of the experimental data.

Results and Analysis

Effects of low phosphorus stress on the growth at different soybean genotype seedling stages

Table 2 showed that the effect of low phosphorus stress on the soybean plant height was less, the average relative height was 96.26%, the difference between different genotypes did not reach significant level, and the variation coefficient achieved its minimum, which was 9.07%. Soybeans, the sensitivity of the relative value of other indicators to the low-phosphorus stress sensitivity both showed differences on genotypes, and their differences had reached a significant level, the variation coefficient was larger, and the order was RRW (26.67%) > RAW (22.68%) > RAPC (24.01%) > RRPC (15.87%). Indicating there were significant inhibition of low phosphorus stress on the RAW, RRW, RAPC and RRPC of different soybean genotypes, in which the effects on the RAW was the greatest.

Table 2 Effects of low phosphorus stress on the growth at different soybean genotype seedling stages %

Genotype	RPH	RAW	RRW	RAPC	RRPC
D3	99.86 a	89.80 ab	79.38 d	80.47 ab	71.19 e
D5	93.74 a	88.11 b	83.87 cd	57.50 d	77.85 c
D17	93.37 a	56.32 f	62.55 f	90.39 a	78.96 bc
D18	105.67 a	79.52 c	84.02 c	68.76 bc	62.78 e
D31	92.50 a	57.04 e	69.04 e	66.75 c	73.68 cd
D34	90.32 a	88.61 b	112.80 a	86.40 a	88.99 a
D37	91.87 a	94.18 a	93.5 b	81.89 ab	81.09 b
D38	102.49 a	70.49 cd	71.09 de	73.35 b	76.61 c
Mean	96.23	78.01	82.03	75.69	76.39
Standard value (SD)	8.73	17.69	21.88	18.17	12.12
Coefficient of variation CV	9.07	22.68	26.67	24.01	15.87

RPH: Relative plant height; RAW: Relative aboveground dry weight; RRW: Relative root dry weight; RAPC: Relative aboveground P concentration; RRPC: Relative root P concentration. The averages followed by the same letter in the same column were not significant at $P < 0.05$ level.

Correlative coefficients among screening criteria soybean genotypes exposed to low phosphorus stress at the seedling stage Correlation analysis results showed that (Table 3) in these five survey indicators, excepted for that the correlation between plant height and the other four indicators had not achieve the significant level, the other indicators all appeared significant or extremely significant positive correlation. In which RRPC was significantly positively correlated with RAW and RAPC, but the correlation coefficients were smaller, which were 0.461 and 0.634. Therefore, RRW, RAW and RAPC could be taken as important screening indicators for the comprehensive evaluation of phosphorus efficiency of soybean seedling, while the RRPC could be used as a supplementary screening indicator.

Table 3 Correlative coefficients among screening criteria soybean genotypes exposed to low phosphorus stress at the seedling stage

	RPH	RAW	RRW	RAPC	RRPC
RPH	1				
RAW	0.407	1			
RRW	0.419	0.958**	1		
RAPC	−0.361	0.854**	0.850**	1	
RRPC	−0.260	0.634*	0.706**	0.461*	1

* represents $P < 0.05$; ** represents $P < 0.01$.

Conclusion and Discussion

Screening and breeding of the phosphorus efficient soybean genotypes have an important significance and economic value to find the potential of soil phosphorus nutrition, maintain good circulation of the soil environment and promote the sustainable grain promotion of soybean. A simple, scientific and effective screening evaluation indicator is an important prerequisite for accurate and rapid screening of efficient phosphorus soybean genotypes. In the low-phosphorus stress environment, the differences on genotype of crops are reflected in the biological traits. At present, the assessment of the tolerance capacity of plant to low phosphorus also appears a lack of unified indexes[9]. Courley thought that the biomass should be used as a standard on the definition of plant nutrition genotype[10]. It was suggested that the relative tiller number[4,11], RAW or relative total biomass[12-13], relative leaf age[14] and so on could be used as ideal indexes for screening of the efficient phosphorus genotypes in rice seedling stage. As for the evaluation of the tolerance capacity of soybean seedlings to low phosphorus, TONG Xue-jun et al thought that the biomass of aboveground parts[15], the absorption of phosphorus of the plant crown[16] could be used as important indexes for the evaluation of the characteristics of phosphorus efficiency of soybean genotypes.

The effect of the external environment on plant stress will be clearly appeared on the corresponding botanical traits through adaptive response, and there are natural differences on the botanical traits among different genotypes of soybean[16]. Therefore, it was believed in this study that the adoption of relative indexes as measurement indexes of the characteristics of phosphorus efficiency was the most simple and intuitive way. The variation of the relative indicators among the genotypes is an important basis to measure whether it was suitable to be a screening index, the more the variation, the more sensitivity of this index to reflect the difference on phosphorus efficiency among genotypes[12]. It was concluded in this study that the effect of low-phosphorus stress on RPH was smaller, and the variation coefficient was smaller, and the correlation of it and other indexes had not achieved the significant level, which was not suitable to be used as a screening index of phosphorus efficiency of soybean seedling; the effect of low-phosphorus stress on RAW, RRW and RAPC was greater, and the variation coefficient was greater, and these indicators all appeared significant or extremely significant positive correlation, therefore, RRW, RAW and RAPC could be taken as important screening indicators for the comprehensive evaluation of phosphorus efficiency of soybean seedling, while the RRPC could be used as a supplementary screening indicator.

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Responsible editor: LI Ting-ting **Responsible proofreader: WU Xiao-yan**

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Responsible editor: FANG Lin **Responsible proofreader: WU Xiao-yan**

羊传染性脓疱病毒 *B2L* 基因片段重组腺病毒载体的构建(摘要)

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[目的]利用腺病毒载体系统构建羊传染性脓疱病毒 *B2L* 基因重组腺病毒载体。
[方法]以从羊传染性脓疱病毒株 JLSY04中提取的基因组 DNA 为模板, PCR 扩增获得 *B2L* 目的基因片段; 然后将 *B2L* 目的基因克隆至 PD-NR-CMV 载体, 筛选阳性克隆获得质粒 CTC572-6, 再将质粒 CTC572-6与腺病毒载体进行同源重组, 筛选阳性克隆, 并进行菌液 PCR、酶切、测序等鉴定。
[结果]经酶切和基因测序等鉴定, 成功构建了携带羊传染性脓疱病毒 *B2L* 基因的重组腺病毒载体 CTC572Ade-30。
[结论]为羊传染性脓疱基因工程疫苗的进一步研究奠定基础。
关键词 羊传染性脓疱病毒; *B2L* 基因; 腺病毒载体

基金项目 吉林省科技发展计划资助项目(20080106)。
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收稿日期 2010-03-01 修回日期 2010-05-11

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大豆苗期耐低磷筛选指标的研究(摘要)

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[目的]探讨简单、可靠的用于大豆苗期磷效率筛选的鉴定指标。
[方法]采用高、低磷土壤盆栽试验: ① 高磷(CK)。施过磷酸钙 3.03 g/kg土, 尿素[$CO(NH_2)_2$] 0.075 g/kg土, 硫酸钾(K_2SO_4) 0.075 g/kg土; ②低磷。只施氮、钾肥, 不施磷肥。对大豆苗期的相对株高(RPH)、相对地上部干重(RAW)、相对根系干重(RRW)、相对地上部磷浓度(RAPC)和相对根部磷浓度(RRPC) 5个指标进行测定。
[结果]相对株高受低磷胁迫影响较小, 变异系数仅为 9.07%, 与其他指标的相关性未达到显著水平; 相对地上部干重、相对根部干重、相对地上部磷浓度和相对根部磷浓度受低磷胁迫的影响较大, 其变异系数也较大, 其顺序为: 相对根部干重(26.67%) > 相对地上部干重(22.68%) > 相对地上部磷浓度(24.015) > 相对根部磷浓度(15.87%), 各指标间的相关系数呈显著或极显著正相关。
[结论]相对地上部干重、相对根部干重和相对地上部磷浓度可以作为综合评价大豆苗期磷效率筛选的重要指标, 相对根部磷浓度可以作为辅助筛选指标。
关键词 大豆; 低磷胁迫; 筛选指标

基金项目 黑龙江省教育厅资助项目"大豆基因型适应低磷土壤的根生物学基础"(10551033); 牡丹江师范学院博士科研启动基金资助项目(MSB200912)。
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收稿日期 2010-02-20 修回日期 2010-03-31