Photooxidation Response as a Method to Screen for Drought Tolerance in Azuki Bean (*Vigna angularis* (Willd.) Ohwi & Ohachi) Germplasm

Chen Xin[1,2] and Peerasak Srinives[1]*

[1]Department of Agronomy, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Kamphaeng Saen, Nakhon Pathom 73140, Thailand

[2]Institute of Vegetable Crops, Jiangsu Academy of Agricultural Sciences, Nanjing, 210014, P.R. China

**Corresponding author. E-mail: agrpss@yahoo.com*

ABSTRACT

One-hundred-and-twenty-one azuki bean (Vigna angularis (Willd.) Owi & Ohashi) accessions were chosen from different regions of China to study the relationship between drought tolerance and photooxidation tolerance. The germplasm was sown in irrigated and non-irrigated cement blocks filled with soil of Kamphaeng Saen Series (a silty-clay loam soil). At flowering, their leaves were excised and submerged in water with low contents of CO2 (~5 µmol/L) and O2 (~350 µmol/L). The leaves were then placed under strong sunshine at the temperature of 30-35°C. The degree of photooxidation in each leaf was determined according to the rate of change in leaf color. The correlation between drought tolerance and photooxidation tolerance of the azuki bean germplasm was r=0.792**. In general, most of the azuki bean accessions were moderately tolerance to photooxidation, while tolerance and non-tolerance varieties were found in less number. Azuki bean germplasm originated from dry area such as Shan Xi and Tian Jin provinces seemed to be more tolerance to drought and photooxidation conditions compared with those from the other provinces. Thus, we recommended that the technique for identifying photooxidation tolerance can be used as a preliminary screening method to identify drought tolerance germplasm in azuki bean.

Key words: Azuki bean, *Vigna angularis* (Willd.) Ohwi & Ohashi, Photooxidation tolerance, Drought tolerance, Preliminary screening

INTRODUCTION

The effect of global warming is alarming agricultural scientists to work more actively towards plant tolerance to drought and heat stresses. For plants grown under strong sunshine, the leaves normally absorb more light energy that is required for electron transport process, resulting in decreasing the activity of Photo System II and photosynthesis as a whole (Yi and Yang, 2005). Under high temperature and drought, the photosynthetic rate was reduced, resulting in yellowing of chlorophyll and leaves, causing the phenomenon called photooxidation. The world's growing areas of legumes, especially azuki bean, mungbean and soybean, often encounter with high temperature, strong sunshine and drought, which make the plants become photooxidized and decreased in yield. Many plant breeders have been looking for drought and photooxidation tolerance germplasm that might be of use in breeding programs (Liu et al., 2005).

For efficient screening of drought tolerance, a quick and easy technique is required to preliminarily screen a large number of materials. Liu and Zhang (1993) used 13 characters to analyze the relationship between drought tolerance and agronomic characters in soybean. Liu and Gai (1989) used controlled irrigation during growth of soybean germplasm for selection of drought tolerance germplasm and found that drought traits responded differently before and after flowering. Although controlled irrigation has been widely used in screening for drought tolerance, the method needs a long time. It is also costly and difficult to do in a large batch of germplasm. A quick test should be developed so that a preliminary screening can be done to reduce the amount of germplasm for further detailed tests.

This experiment was conducted to determine the relationship between photooxidation and drought tolerance so that an easy and quick method can be established to select for drought tolerance germplasm in azuki bean.

MATERIALS AND METHODS

This experiment was conducted in dry season of 2008 in crossing blocks of the Tropical Vegetable Research and Development Center, Kasetsart University, Kamphaeng Saen, Nakhon Pathom Province, Thailand. One-hundred-and-twenty-one azuki bean accessions were used in this study. Among them, 109 were obtained from different regions of mainland China, 12 were obtained from Taiwan. The study was conducted in two experiments, one was designed to observe drought tolerance and the other was to observe photooxidation tolerance.

Determination of drought tolerance index

All azuki bean accessions were sown in each of the two cement blocks filled with Kampaeng Saen Series soil (a silty-clay loam soil). Each block was sown to the same 121 accessions. The first block was fully irrigated while the second was irrigated up to flowering to observe for drought stress. Fifteen days after planting, the germplasm was rated for drought tolerance in 4 scales. The accessions with the scale of 1 showed all plants without wilting sign on the leaves, the scale of 2 was given to the accessions with at least 75% of the leaves showed no wilting, the scale of 3 was given to the accessions with 25-75% of the leaves showed no wilting and the scale of 4 was given to the accessions with < 25% of the leaves showed no wilting. In addition, plant height, number of branches per plant and fresh weight (g/plant) of each accession were measured from all accessions in both blocks, and changed into the scale index of 0 to 4 based on the relative value obtained from irrigated and non-irrigated blocks. For a particular trait, the accessions with the scale of 0 showed relative value of greater than 1.00, those with the scale of 1 had the relative value of 0.76-1.00, the scale of 2 had the value of 0.51-0.75, the scale of 3 had the value of 0.26-0.50 and the scale of 4 had the value of 0.25 or less. Then, a drought tolerance index of each accession was determined as a composite index as follows (Liu et al., 2005):

Drought tolerance index = 1/4(drought scale index + plant height index + number of branches index + fresh weight index). Since it is possible that some accessions gave higher values of some traits under drought condition, they can have the composite drought tolerance index of less than one. However, the composite cannot exceed 4.0. Thus, the drought tolerance index has the theoretical values ranging from 0 to 4. The lower the index, the more the tolerance. Roughly, the accessions with the drought tolerance index less than 1.00 belonged to the drought tolerance group, the tolerance index from 1.00 to 1.99 belonged to the moderately drought tolerance group, the index from 2.00 to 2.99 belonged to the moderately drought susceptible group, while the index of 3.00 and above belonged to the drought susceptible group.

Determination of photooxidation tolerance scale

The same set of accessions was sown in an experimental field using the spacing of 25 × 50 cm, with one plant per hill. There were 16 plants in each accession. The middle leaflet of the 3rd leaf from the top was put in a transparent plastic box, using 6 plants per accession. The leaves were kept to remain fresh by foggy spraying of water and covering the box with a plastic sheet. The box was put in the sunshine for 2 days to accelerate photooxidation process. Reaction to photooxidation was again classified into 4 scales (Liu et al., 2005). The scale of 1 was given to the accessions that showed >75% of the leaf area still green, the scale of 2 was given when 50-75% of the leaf area was still green, the scale of 3 was given when 25-49% of the leaf area was still green, while the scale of 4 was given when < 25% of the leaf area was still green. Then, the average scale across 6 leaves was used as the photooxidation index of the accession.

A scaling example is given in Figure1 which showed that photooxidation is a quantitative character and should be scaled such that a wider range of reaction can be covered as the scale of 1 to 4 used in this study. The relationship between drought tolerance index and photooxidation tolerance scale was determined from the correlation coefficient between both measurements of tolerance across the 121 azuki bean accessions.

Figure 1. Reaction to photooxidation of different leaves upon exposing to sunshine; the scale was given as 4, 2 and 1, from left to right.

Statistical analysis

The relationship between drought tolerance index and photooxidation tolerance scale was presented in a regression line, using the index as Y-variable and the scale as X- variable. The coefficient of determination (R^2) was also calculated to quantify the variation in drought tolerance index as explained by the photooxidation tolerance scale (Steel and Torrie, 1980).

RESULTS

Drought response in azuki bean

The azuki bean germplasm showed different reaction to drought. Five accessions can be considered belonging to the very tolerance group with the composite indices of less than 0.50. Most of the accessions in this study belonged to the more drought susceptible groups (scale 3.50 and above). The five drought tolerance accessions were Jingchi821, Shanzi49, Tianchi03, Tianchi71 and Jinxiaodou0521. Three of them were from Shan Xi province while two of them came from Tianjin city. The areas planted to azuki bean in these two provinces are located in yellow-soil altiplano where the area is dry and the soil is sandy. The azuki bean accessions also showed different expression in agronomic characters. For instance, Tong19 was classified as moderately drought susceptible group, but its height was only slightly changed upon stopping of irrigation as compared with the irrigated plants, while the branches and the fresh weight decreased drastically. The accession Su0021 showed a little change in number of branches upon exposing to drought while the height and fresh weight decreased significantly. Thus, it is clear from this study that drought tolerance germplasm can be of more use when agronomic characters are co-assessed and considered as shown in the composite indices used in our study.

Photooxidation response in azuki bean

There were 8 azuki bean accessions which were rated at the scale of 1 in terms of photooxidation (Figure 2). They were the above-mentioned 5 accessions plus Suzi98, Liaozi921 and Liaozi190. In total, 6.5% of the germplasm belonged to the tolerance group (rated 1), 8% belonged to the susceptible group (rated 4) and 85.5% belonged to the intermediate groups (rated 2 and 3).

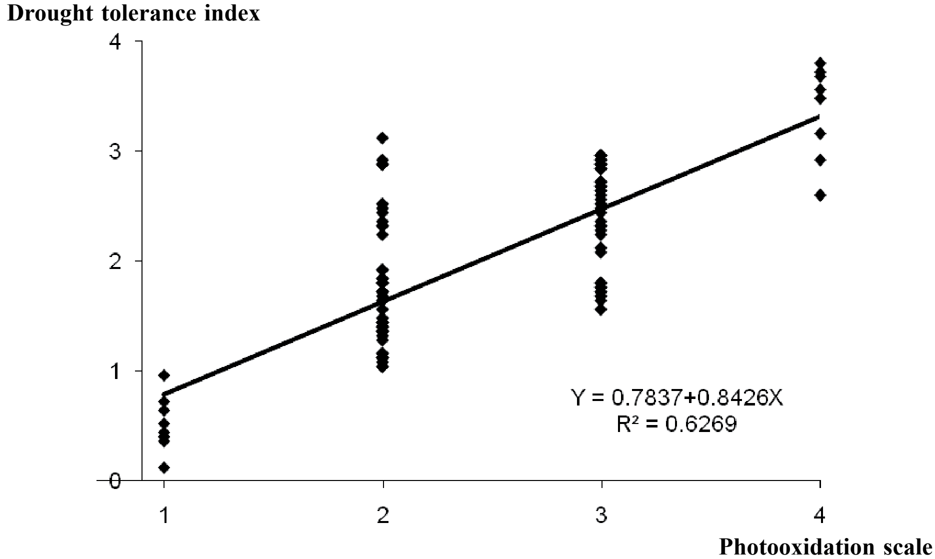

Figure 2. Relationship between leaf photooxidation scale and drought tolerance index in 121 azuki bean accessions.

Correlation between drought tolerance and photooxidation tolerance

The correlation coefficient between drought tolerance index and photooxidation scale among 121 azuki bean accessions was highly significant (r=0.792** at df = 119). This means that both methods showed a high positive relationship among each other. The regression equation of photooxidation on drought tolerance index is shown in Figure 2 as Y=0.7837+0.8426X. A high coefficient of determination (R^2 = 0.6269) revealed that variation in drought tolerance index can be largely explained by the simple photooxidation tolerance scale.

DISCUSSION

During the past two decades, a number of experiments have been conducted on drought tolerance in several crops. Some scientists approached the problem by measuring response to photooxidation (Peng et al., 1998; Li et al., 1999; Ji et al., 2001, Liu et al., 2005). The principle behind this idea is based on the fact that stomata on the leaves are open and allow oxygen to pass through during photosynthesis. Under sunshine, the excess photons combine with oxygen and create active oxygen species in the leaves which then impair with photosynthesis process (Gu et al., 1998; Shao et al., 1998; Peng et al., 2000). Major forms of active oxygen species in plants are $.O_2$-, H_2O_2, .OH, $^1O_2$, etc. (Tawa and Sakurai, 1997; Liszkay et al., 2004). These active oxygen species are harmful to chlorophyll and membrane, and thus cause yellowing of the leaves (Feng et al., 2005), and finally affect crop yield. Although scientists can use traditional water control method to identify drought tolerance germplasm, the method is expensive and cannot be used in a large array of germplasm. In our experiment, a fast and cheap method was introduced as an alternative by putting the azuki bean leaves under the sunshine to encourage active oxygen to attack the photosynthesis pathway, and cause decreasing in photosynthesis pigments and finally lead to yellowing and whitening of the leaves (Gu et al., 1999; Zhu and Wang, 2003; Li et al., 2005).

In the future, observations should be done on morphological traits which may have good association with drought tolerance such as root system, plant type and etc. to support those drought tolerance accessions. Observations should also be done on leaf characters which have good association with photooxidation such as thickness of leaf, leaf shape and etc. to support those photooxidation tolerance accessions. This can bring to the selection of the parental lines used in breeding for drought tolerance in azuki bean.

CONCLUSION

An environment can be created to induce water stress after flowering in azuki bean to determine drought tolerance indices. The normal leaves were subjected to sunshine and high temperature, which induced the closure of the stomata, resulted in low CO_2 and O_2 contents, depressed photosynthetic pigments and finally impaired with the photosynthetic system. This concept was well validated in this experiment, as the relationship between the drought tolerance index and photooxidation tolerance scale was high. This simple method can be used to identify drought tolerance germplasm in azuki bean, and can also be extended to apply to the other crops as well.

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