# Selection of Heat Tolerant Lablab

District Agricultural Research and Extension Station

Bing-Yun Tsai

## Abstract

The heat tolerance of 44 WorldVeg accessions of *Lablab purpureus* at vegetative stage from WorldVeg gene bank was examined in terms of high cell membrane stability and chlorophyll fluorescence as two heat-stress tolerant traits at vegetative stage. Relative injury index, is a parameter, which reflects cell membrane stability. All accessions with an injury index below 50% were selected as promising for this trait. The photosynthetic efficiency ratio is a parameter, which reflects chlorophyll fluorescence. All accessions with an injury index above 35% were selected as promising for this trait. Six accession were considered as heat tolerant accession. However, no correlation was found between relative injury index and photosynthesis efficiency ratio.

## Introduction

*Lablab purpureus*, typically known as lablab bean, hyacinth bean, dolichos bean or Indian bean, is cultivated for various purposes such as vegetable, livestock fodder, or cover crop for soil improvement and soil protection. It has considerable adaptation to acidity, low soil phosphorous and drought (Mugwira & Haque, 1993; Robotham & Chapman, 2017). Global warming is rising average and extreme temperature causing heat stress to plants (IPCC, 2014). High temperatures cause chlorosis, growth redundancy, wilting, tissue necrosis, resulting in lower yields or even complete crop losses.

In Taiwan eastern lowlands, heat stress limits lablab production in the summer time between May and August. To improve production stability in summer, farmers need heat tolerant varieties. However, the lack of lablab pre-breeding research makes it difficult to identify desirable genotypes in lablab. Physiological and biochemical trait screening is desirable to select promising accessions for breeding.

This study examines heat tolerance at seedling stage using two indicators of heat tolerance: 1) leaf electrolyte leakage as a measurement of Cellular Membrane Stability (CMS); and 2) the chlorophyll fluorescence parameter, Fv/Fm. CMS measured by electrolyte leakage is a convenient method that widely used to detect heat tolerance in cereal, vegetable, fruit tree and ornamental plant (Ahren & Ingram, 1988; Thiaw & Hall, 2004; Yeh & Hsu, 2004). At the same time, chlorophyll

fluorescence is a sensitive and rapid tool to detect photosynthesis efficiency of photosystem II. Forty-four lablab accessions from WorldVeg gene bank have been evaluated to select heat tolerant germplasm for lablab breeding.

**Cell Membrane Stability (CMS).** Injuries of heat include protein degradation or aggregation, accumulation of toxic compounds and reactive oxygen species (ROS), and increased fluidity of membrane. CMS measures electrolyte leakage from leaf samples at high temperature. It is a rapid and sensitive screening technique for heat stress tolerance. This method is based on cell membrane permeability, which increases at higher temperatures. At higher temperature, electrolyte leaks out from leaf cytoplasm to the surrounding solution in which the leaf samples are bathed. Accessions with high heat tolerance keep low levels of cell membrane permeability at high temperatures. The concentration of electrolytes in the solution can be measured by electro conductivity. CMS has proved to be an efficient method to detect the variance of heat tolerance in wheat, tomato and pepper (Blum & Ebercon, 1981; Camejo et al., 2005; Zhanget al., 2001).

**Chlorophyll fluorescence parameter, Fv/Fm.** There are three major site particularly sensitive to heat in photosynthetic machinery. They are mainly in photosystem II which are oxygen evolving complex, ATP generation and carbon assimilation process. At moderate elavated temperature, D1 protein of oxygen evolving complex would damaged by ROS and inhibit repair mechanism (Allakhverdiev et al., 2008). At high temperature, ribulose-1,5-bisphosphate carboxylase/ oxygenase with low affinity of the enzyme for $CO_2$ and its dual nature as an oxygenase limit net photosynthesis. At the same time, stomata closure reduce gas exchange, causing $CO_2$ concentration even lower. Chlorophyll fluorescence is a non-destructive and efficient analysis to measure photosynthesis efficiency at high temperatures. Additionally, chlorophyll fluorescence reflects the real-time stage of photosynthetic systems. Chlorophyll fluorescence supported by flux theory have been suggested to represent photosynthesis efficiency in photosystem II. Fv/Fm, a chlorophyll fluorescence parameters that indicate maximum quantum yield of PSII primary photochemistry in the dark-adapted state, is the most common parameter describing photosynthesis efficiency. It has been used to check heat tolerance of maize, pepper and snap bean in previous studies (Sinasawat et al., 2004; Costa et al., 2003; Wang and Tseng, 2010).

## Material and methods

**Seedling preparation.** The experiment contained 44 accessions (Table 1) from WorldVeg gene bank, which were selected from different countries to reflect its geographic diversity. The accessions were planted in 3-inch pot containing peat moss and coconut fiber (King root

substrate No. 1, Dayi Agritech Co., Pintung). Seedlings were grown in net house at mean temperature of 27.0°C under light intensity of 16410 lux.

**Cell Membrane Stability (CMS).** Leaf samples were extracted from fully expanded primary leaves before shoot tip internode elongated. Three plants were analyzed out per accession. Each leaf sample consisted of a paired set of five 6-mm-diameter leaf discs. Leaf discs were washed carefully with distil water in petri dishes to prevent other damage. Then these leaf discs were placed into test tube containing 1 ml distilled water covered by aluminum foil and put into a heated and circulated water bath at 30°C and 50°C for 30 min. Fourteen ml of room-temperature distilled water was added after heating. Electro conductance (EC) of these leaf discs was measured after under two treatments: treatment 1) 12 hours resting; and treatment 2) 12 hours resting followed by sterilization at 121℃ and 1.2 kg· $cm^{-2}$ for 20 minutes. Samples were cooled up to room temperature before final measurement by CM31P portable electric conductivity meter (DKK-TOA Co., Tokyo). Relative injury (RI) value was calculated as follow: $\mathrm{RI}(\%) = \{1 - [1 - (\mathrm{T1}/\mathrm{T2})]/[1 - \mathrm{C1}/\mathrm{C2}]\} \times 100\%$, where T and C refers to EC value for treatment and control, and 1 and 2 refer to treatment 1 (only 12 hours rest) and treatment 2 (resting and sterilization), respectively.

**Photosynthesis efficiency.** Leaf samples were extracted from fully expanded primary leaves before shoot tip elongated and cut into half along with vein. Three plants were analyzed per accession. Each pair set of half leaf, placed in petri dish and wrapped by Para film, would heated in circulated water bath at 30°C and 50°C for 30 min respectively. After cooled down, samples were covered by black flannel for at least 30 minutes as dark-adaptation. Measure leaf chlorophyll fluorescence under black flannel by MINI-PAM photosynthesis yield analyzer (Heinz Walz Co., Effeltrich). Relative photosynthesis efficiency was calculated as follow: $\text{Relative photosynthesis efficiency}\ (\%) = \mathrm{P50}/\mathrm{P30} \times 100\%$, where P refers to Fv/Fm, and 50 and 30 refers to treat temperature.

**Statistical analysis**. Experiment design is completely randomized design. The SAS® 9.4 software was used for statistical analysis. The data were subjected to analysis of variance (ANOVA). Means comparison was performed using Duncan's Multiple Range Test. Significance was defined as $P<0.05$.

## Results

**Cell Membrane Stability (CMS) under heat stress.** In the 50℃ heat treatment, lablab accessions show a wide range of cell membrane thermal stability between 30.3-82.4% (Figure 1, Table 2). The result shows that heat tolerance in lablab leaves between accessions is detectable by CMS. 50% relative injury was taken as a threshold. Accessions with index values lower than this point

were considered as heat tolerant. On the basis of this threshold, twelve accessions were selected as promising accessions for heat tolerance (Table 2).

**Photosynthesis efficiency under heat stress.** Photosynthetic efficiency ratio varied between 19.9% to 49.4% (Figure 2; Table 2). For photosynthesis efficiency ratio, the higher the value the more efficiency remain after heat treatment. The average ratio of 34.1% was used as threshold. On the basis of this threshold, 23 accessions were identified as heat tolerant.

**Relationship between CMS and photosynthesis efficiency.** There is no correlation between CMS and photosynthesis efficiency. By selection of 50% relative injury and 34.1% photosynthetic efficiency ratio, there were only 6 accessions selected, which were heat tolerant following both heat stress selection approaches. Fifteen accessions were considered susceptible to heat tolerant. Interestingly, 23 accessions reveal tolerance in only one parameter but not the other. For example, VI055928 (L39) was the best performing accessions for photosynthetic efficiency, with a ratio of 49.4%. However this accessions, performed poorly for the indicator of relative injury; with a percentage 68.5% relative injury, its relative injury was high.

**Geographic patterns.** The heat tolerant Accessions are from Bangladesh, Lao People's Democratic Republic, Australia, Uzbekistan and Ethiopia. No geographic patterns for heat tolerance lablab germplasm could be found in this study.

## Discussion

Although CMS and chlorophyll fluorescence are commonly used to detect heat tolerance in various crops, there is no correlation between these parameters in this research. Different tolerance mechanism crops took might be the reason. Both membrane and photosynthesis would be challenged by ROS, protein misfolded and enzyme activities affection or binding site affinity changed. The tolerance mechanism including heat shock protein, unfold protein response, reactive oxygen scavenger and so on (Wahid *et al*., 2007). However, CMS could be influenced by protein channel and membrane composition, like saturate and unsaturated lipid acid or cholesterol. On the other hand, it has been reported that heat could affect photosynthesis by stomata closure, unbalance electron transport, ribulose-1,5-bisphosphate carboxylase/ oxygenase inactivation and other protein misfolded (Crafts-Brandner & Law, 2000; Sinsawat et al., 2004). For CMS and chlorophyll fluorescence represent to the damage of different cell components and different repair pathway, the result indicate that there are several heat-tolerance mechanism in lablab.

The results show that variation within accessions was larger compared with the difference between accessions. It could be due to the heterozygosity within genebank accessions is high

because most are local cultivars. For further breeding material selection, purification should be done. On the other hand, CMS has been reported not very sensitive in cabbage and soybean (Nyarko *et al*., 2008; Martineau *et al*, 1979) and been suggested using at least 6 plants per accessions.

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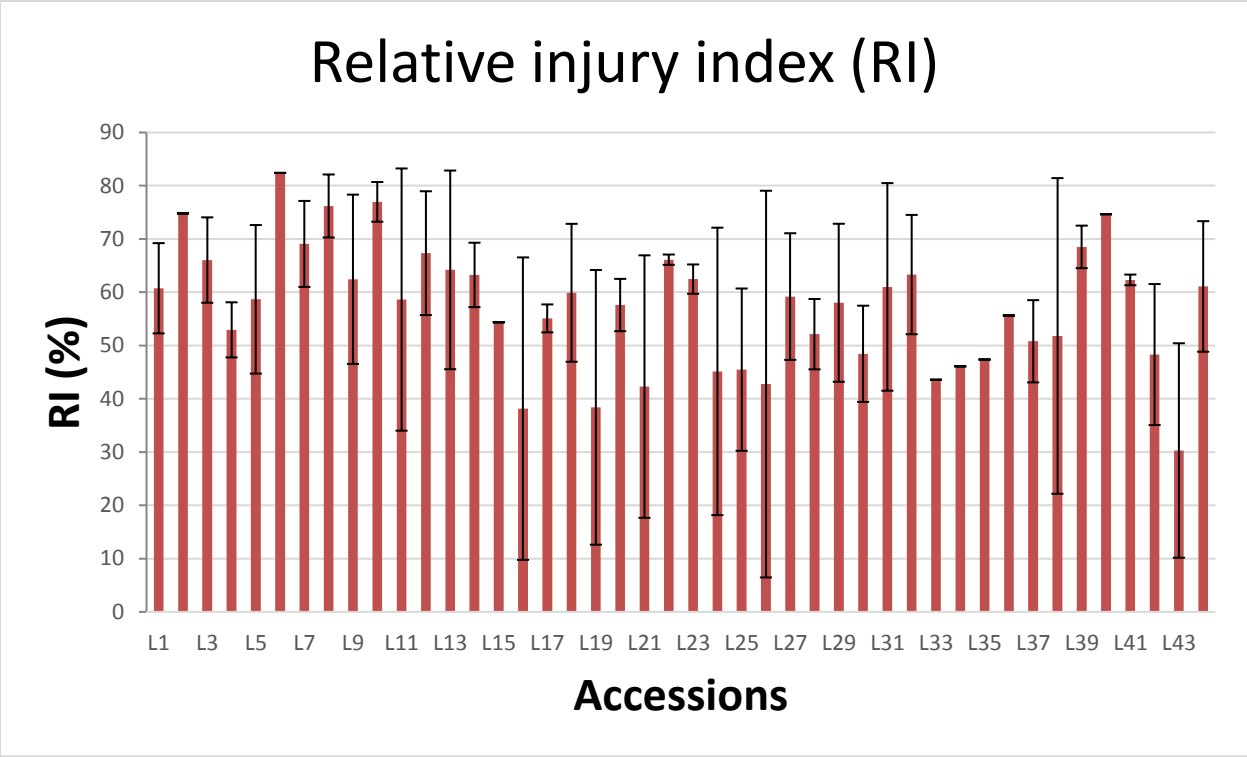


Fig. 1 Relative injury index of lablab primary leaves treated under 50℃ for 30 min. Accessions with low values show high heat stress tolerance following this method.

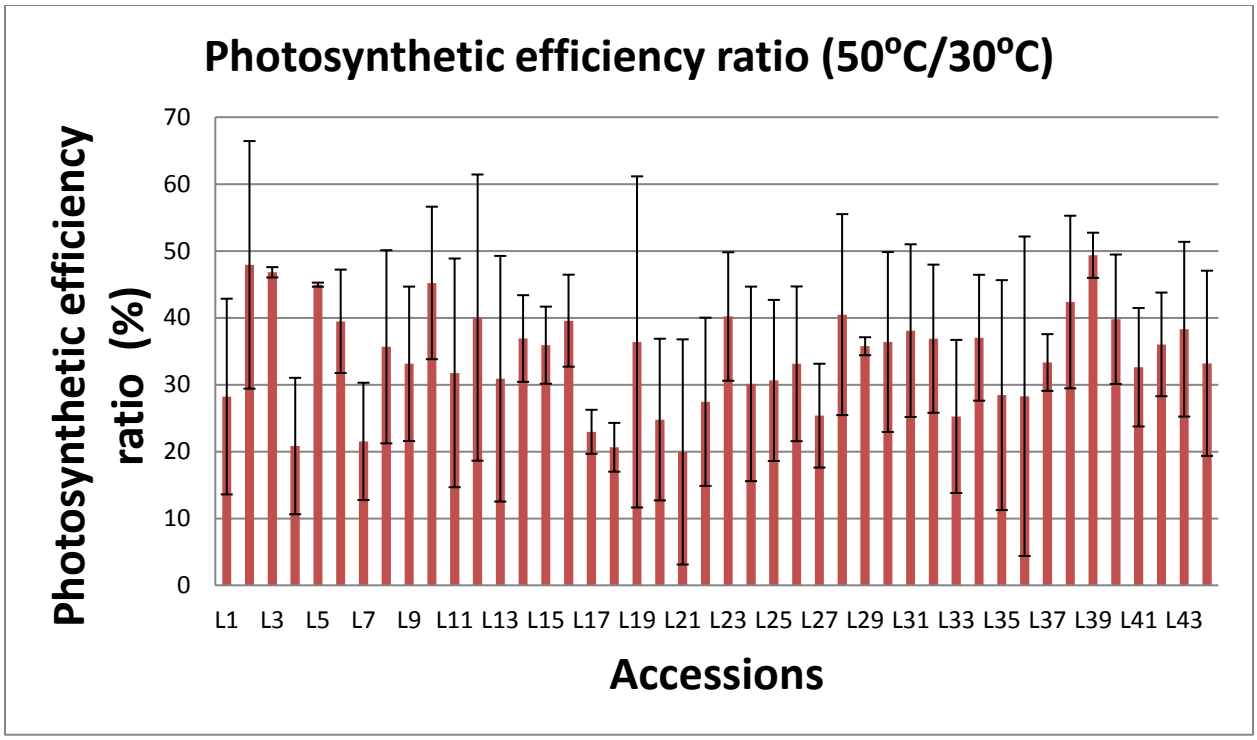


Fig. 2 Photosynthetic efficiency ratio of lablab primary leaves treated under 50℃ for 30 min. Accessions with high values show high heat stress tolerance following this method.

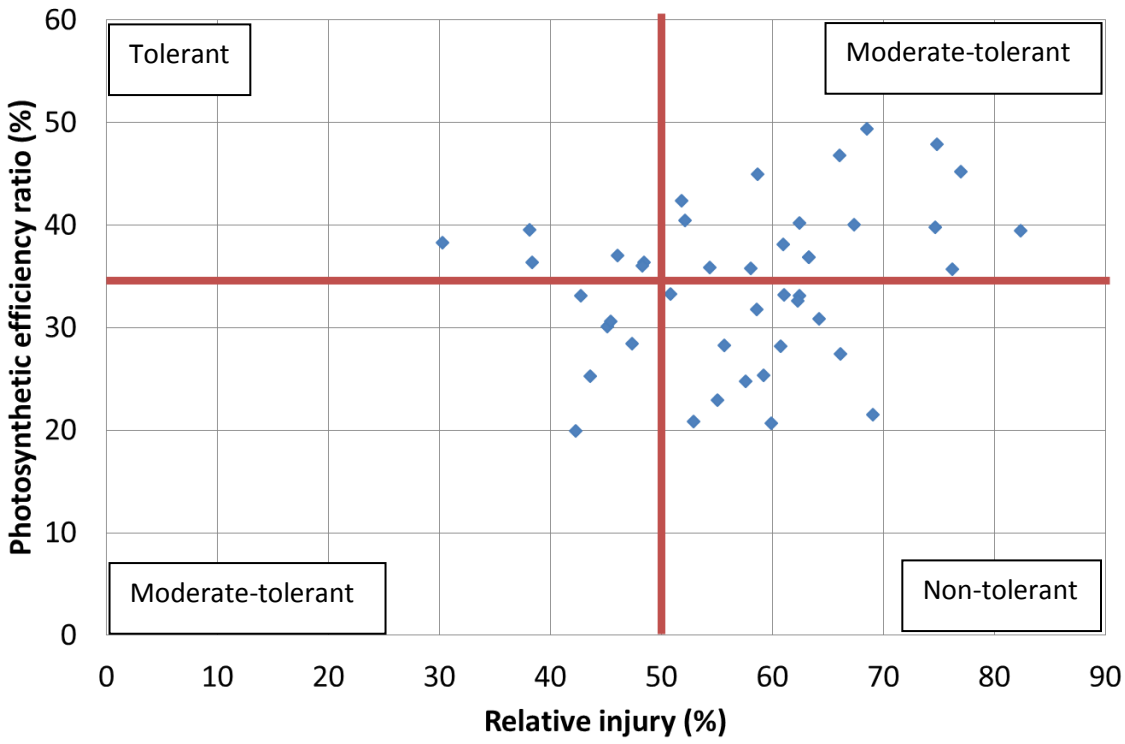


Fig. 3 Relationship between Cell Membrane Stability (CMS) and photosynthesis efficiency. The accessions in the upper-left square are heat tolerant according to these two methods. The accessions in the upper-right and lower-left square are heat tolerant according to one of the two methods. Accessions in the lower-right square are susceptible to heat according to the two methods.

Table 1 Passport data of 44 accessions of lablab from WorldVeg gene bank.

| Test code | Accession No. | Cultivar | Collected Nation |
|---|---|---|---|
| L1 | VI033686 | KHALO SIMI | Nepal |
| L2 | VI033689 | SIMBI | Nepal |
| L3 | VI033696 | BARAMASI SIMI | Nepal |
| L4 | VI033742 | PARDA | Philippines |
| L5 | VI034562 | | Malaysia |
| L6 | VI036241 | BATAO | Philippines |
| L7 | VI036243 | PUTI | Philippines |
| L8 | VI039981 | THUA PAEP SON | Thailand |
| L9 | VI040057 | THUA PAEP | Thailand |
| L10 | VI040619 | THUAPAEB | Thailand |
| L11 | VI040869 | THUA-RHA | Thailand |
| L12 | VI043319 | | Indonesia |
| L13 | VI043321 | | Indonesia |
| L14 | VI046639 | DAU VAN | Viet Nam |
| L15 | VI047215-A | MAK PEP-LE | Lao People's Democratic Republic |
| L16 | VI047701 | SHEEM | Bangladesh |
| L17 | VI047741 | SHIM | Bangladesh |
| L18 | VI047742 | SHIM (SITAKUNDU TYPE) | Bangladesh |
| L19 | VI047925 | CHAPTA SHEEM | Bangladesh |
| L20 | VI048064 | KARTIKA SHEEM | Bangladesh |
| L21 | VI048216 | CHURI CHAI/ BATA CHAI/ LAL CHAI | Bangladesh |
| L22 | VI048224 | JAT CHAI | Bangladesh |
| L23 | VI048353 | DAU VAN DO | Viet Nam |
| L24 | VI048595 | | Taiwan |
| L25 | VI049394 | THAU PAB | Thailand |
| L26 | VI054751 | PD-12 | India |
| L27 | VI054753 | PER-12 | India |
| L28 | VI054754 | V-10 | India |
| L29 | VI054755 | IL-41089 | India |
| L30 | VI054852 | MAK PEP | Lao People's Democratic Republic |
| L31 | VI055040 | | Malaysia |

| | | | |
|---|---|---|---|
| L32 | VI055218 | KACANG SEPAT HIJAU | Malaysia |
| L33 | VI055306 | | Australia |
| L34 | VI055307-A | | Australia |
| L35 | VI055308 | | Ethiopia |
| L36 | VI055312-A | | Australia |
| L37 | VI055316 | | Ethiopia |
| L38 | VI055603 | THUA PAEH | Lao People's Democratic Republic |
| L39 | VI055928 | MAK PEP | Lao People's Democratic Republic |
| L40 | VI056060 | | Cambodia |
| L41 | VI056099 | | Cambodia |
| L42 | VI056369 | VYUN | Uzbekistan |
| L43 | VI056723 | | Ethiopia |
| L44 | VI057087 | | Cambodia |

Table 2 Mean values and selection of the 44 lablab accessions

| Test code | Accession No. | Relative injury index (%) | Photosynthetic efficiency ratio (%) | Heat tolerance selection |
|---|---|---|---|---|
| L1 | VI033686 | 60.8 abcdefgh | 28.2 abcdef | Non-tolerant |
| L2 | VI033689 | 74.8 abc | 47.9 def | Moderate tolerant |
| L3 | VI033696 | 66.1 abcdefg | 46.8 cdef | Moderate tolerant |
| L4 | VI033742 | 52.9 cdefghi | 20.8 abc | Non-tolerant |
| L5 | VI034562 | 58.7 abcdefghi | 45.0 abcdef | Moderate tolerant |
| L6 | VI036241 | 82.4 a | 39.5 abcdef | Moderate tolerant |
| L7 | VI036243 | 69.1 abcde | 21.5ab | Non-tolerant |
| L8 | VI039981 | 76.2 abc | 35.7 abcdef | Moderate tolerant |
| L9 | VI040057 | 62.4 abcdefgh | 33.1 abcdef | Non-tolerant |
| L10 | VI040619 | 77.0 ab | 45.2 ef | Moderate tolerant |
| L11 | VI040869 | 58.6 abcdefghi | 31.8 abcdef | Non-tolerant |
| L12 | VI043319 | 67.3 abcdef | 40.0 abcdef | Moderate tolerant |
| L13 | VI043321 | 64.2 abcdefgh | 30.9 abcde | Non-tolerant |
| L14 | VI046639 | 63.3 abcdefgh | 36.9 abcdef | Moderate tolerant |
| L15 | VI047215-A | 54.4 bcdefghi | 35.9 bcdef | Moderate tolerant |
| L16 | VI047701 | 38.2 fghi | 39.6 abcdef | Tolerant |
| L17 | VI047741 | 55.1 bcdefghi | 23.0 abcde | Non-tolerant |
| L18 | VI047742 | 59.9 abcdefgh | 20.6a | Non-tolerant |
| L19 | VI047925 | 38.4 hi | 36.4 abcdef | Tolerant |
| L20 | VI048064 | 57.6 abcdefghi | 24.8 abc | Non-tolerant |
| L21 | VI048216 | 42.3 ghi | 19.9 abcd | Moderate tolerant |
| L22 | VI048224 | 66.1 abcdefg | 27.4 abcdef | Non-tolerant |
| L23 | VI048353 | 62.5 abcdefgh | 40.2 abcdef | Moderate tolerant |
| L24 | VI048595 | 45.1 defghi | 30.1 abcdef | Moderate tolerant |
| L25 | VI049394 | 45.5 defghi | 30.6 abcdef | Moderate tolerant |
| L26 | VI054751 | 42.8 defghi | 33.1 abcdef | Moderate tolerant |
| L27 | VI054753 | 59.2 abcdefgh | 25.4 abcdef | Non-tolerant |
| L28 | VI054754 | 52.1 cdefghi | 40.5 f | Moderate tolerant |
| L29 | VI054755 | 58.0 abcdefghi | 35.8 abcdef | Moderate tolerant |
| L30 | VI054852 | 48.5 cdefghi | 36.4 abcdef | Tolerant |
| L31 | VI055040 | 61.0 bcdefghi | 38.1 bcdef | Moderate tolerant |
| L32 | VI055218 | 63.3 abcdefgh | 36.9 abcdef | Moderate tolerant |

| | | | | |
|---|---|---|---|---|
| L33 | VI055306 | 43.6 defghi | 25.3 abcdef | Moderate tolerant |
| L34 | VI055307-A | 46.1 defghi | 37.0 abcdef | Tolerant |
| L35 | VI055308 | 47.4 defghi | 28.4 abcdef | Moderate tolerant |
| L36 | VI055312-A | 55.6 bcdefghi | 28.3 bcdef | Non-tolerant |
| L37 | VI055316 | 50.8 bcdefghi | 33.3 bcdef | Non-tolerant |
| L38 | VI055603 | 51.8 bcdefghi | 42.4 bcdef | Moderate tolerant |
| L39 | VI055928 | 68.5 abcde | 49.4 ef | Moderate tolerant |
| L40 | VI056060 | 74.6 abcd | 39.8 bcdef | Moderate tolerant |
| L41 | VI056099 | 62.3 abcdefgh | 32.6 abcdef | Non-tolerant |
| L42 | VI056369 | 48.3 cdefghi | 36.0 abcdef | Tolerant |
| L43 | VI056723 | 30.3 i | 38.3 abcdef | Tolerant |
| L44 | VI057087 | 61.1 abcdefgh | 33.2 abcdef | Non-tolerant |

Suggestion for future cooperation：

1. Strengthen cooperation with each other. WorldVeg is an international research institute. Care about stable production and nutrient balance in the world. Corporate with WorldVeg not only broad the thinking, but also provide the information about south bound country and Africa demand. WorldVeg is an important partner for Taiwan to internationalize our garniture industry.

2. Cooperate with gene bank in WorldVeg. WorldVeg collect vegetable germplasm all around the world. There are many germplasm materials for further breeding program. Needless to say they are doing native vegetables germplasm analysis and develop core collection for mung bean.

3. Invite researcher in WorldVeg to research institute in Taiwan for shot-term exchange. Researcher in WorldVeg have mention about they are willing to cooperate with research institute in Taiwan. However, the developing and research goal and related resource are not clearly understand. To strengthen the cooperation, they are willing to have a short-term exchange to different research institute.