Mycopath (2016) 14(1&2): 1-7 **Research Article**

Response of *Vigna radiata* (L.) Wilczek genotypes to charcoal rot disease

***Kashif Ali Khan, Amna Shoaib and Sundus Akhtar**

Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan
**Corresponding author's email: rana13tda@yahoo.com*

Abstract

Charcoal rot disease of mung bean [*Vigna radiata* (L.) Wilczek] caused by *Macrophomina phaseolina* (Tassi) Goid, is one of the highly destructive diseases of the crop growing arid region of the Pakistan. Twenty six varieties of mung bean were screened out for their resistance against *M. phaseolina* in artificially inoculated sandy loam soil collected from the District Bhakkar, Punjab. The experiment was conducted in pots kept in a completely randomized deigned with three replications for 60 days and the acquired data were analyzed statistically. Mung bean genotypes were categorized on the basis of disease severity, plant mortality and growth inhibition index (GII). Results showed that among the 26 genotypes, 2 (MNUYT-317 and NM-2011) were found highly resistant, 10 moderately resistant (Mung-12004, MNUYT-317, Mung-12007, MNUYT-201, MNUYT-219, AZRI-2006, Mung-12002, MNUYT-318, MNUYT-207 and MNUYT-107), 7 susceptible (MNUYT-103, MNUYT-11, MNUYT-301, MNUYT-16 MNUYT-7, MNUYT-109 and MNUYT-312) and rest of 7 (MNUYT-102, FS-0318, MNUYT-210, MNUYT-118, MNUYT-204, MNUYT-18 and MNUYT-105) as highly susceptible. Screening of mung bean for identification of resistance against charcoal rot disease in potted soil is found be appropriate, short term and efficient method prior to release as approved variety in the market to avoid field losses.

Keywords: Green gram, Charcoal rot, *Macrophomina phaseolina*, Resistance

Introduction

Mung bean or green gram belongs to family Fabaceae is one of the most important traditional pulse crops characterized by a tremendous protein content along with excellent and adequate quantities of sulfur-containing amino acids (Mensah and Ihenyen, 2009). Like other countries of the world, mung bean is one of the important pulse after chick pea in Pakistan cultivated on an area of 130.9 thousand hectares with a net production of 93.9 thousand tones (Anonymous, 2013). Layyah, Bhakkar, Mianwali and Rawalpindi are major mung bean cultivating arid areas in Pakistan. Although cultivated area, production, and per acre yield of mung bean has increased during last few years but yield per acre in the country is still marginal (Hanif *et al*., 2013).

Among essential problems encountered by mung bean, charcoal rot caused by *Macrophomina phaseolina* is significant disease in reducing yield of economical important crop especially in arid regions of the world (Khan and Shuaib, 2007). The fungus ability to adapt itself according to prevailing environmental condition in soil through great level of changes in morphology, biochemistry and pathology make it the most aggressive pathogen. Whereas, the fungus ability to produce microsclerotia in saprophytic phase and pycnidia in pathogenic phase encounter it to act as non-specific pathogen with massive yield loss (Beas-Fernández *et al*., 2006). Therefore, up till now more than 500 plant species of 75 families have been reported to be infected by this fungus (Rayatpanah and Dalili, 2012). In addition to that, the fungus exhibits potential to attack plants on almost every growth stages and may cause death of young seedling due to formation of dark irregular lesions on the epicotyls and hypocotyls that extends to the cotyledons. The adult plant dies due to seedling blight; stem and pod rot followed by blockage of xylem vessels (Beas-Fernández *et al.*, 2006). The presence of the pathogen in seed poses a serious risk to some overseas sprouting seed markets because disease may cause up to 100% yield losses (Iqbal *et al*., 2010).

Generally, *M. phaseolina* found to be comparatively hard pathogen to control and disease management strategies applied as cultural, chemical or biological fail to provide desirable results (Abdel-Kader *et al*., 2010). Screening of mung bean germplasm against *M. phaseolina* is imperative to identify resistance genotypes. Many resistance lines have been screened by several workers (Khan and Shuaib, 2007; Hasseb *et al*., 2013; Atiq *et al*., 2014) and new germplasms are being introduced in the market due to increasing demand of pulses. Therefore, the present study was aimed to screen 26 mung bean genotypes against *M. phaseolina* in response to charcoal rot disease in sandy loam soil from district Bhakkar, Punjab, Pakistan.

Materials and Methods

Procurement and culturing of the pathogen

Pure culture of *M. phaseolina* (FCBP # 0751) was procured from First Fungal Culture Bank of Pakistan, Institute of Agricultural Sciences (IAGS),

University of the Punjab, Lahore. For the experiment, fungal culture was sub-cultured on 2% malt extract agar (2 g malt extract, 2 g agar and 100 mL distilled water) in Petri plates incubated at 28 ± 2 °C for 7 days. For the pathogenicity trail, mass culture of the fungus was prepared on sorghum seeds. Plastic bags filled with pre boiled sorghum seed (20 g) were autoclaved at 121 °C, 15 psi for 15 minutes, and were inoculated under aseptic condition with inoculum (5 mm disc) of the fungus taken from 7-days old culture. Inoculated bags were incubated in incubator at 28 ± 2 °C for 12 days. After incubation period, when mycelial completely ramified the substrate, spore count in each bag was measured with haemocytometer.

Procurement of mung bean genotypes

Twenty six mung bean varieties were collected from Arid Zone Research Institute, Bhakkar and Ayub Agricultural Research Institute, Faisalabad. Healthy seeds were surface sterilized with 2% sodium hypochlorite for two minutes followed by 3 consecutive washings in distilled sterilized water and spread over blotting paper to dry the seeds.

Pot experiment

The experiment was conducted in plastic pots kept in tunnel at the experimental research area of IAGS during the year 2013 with sandy loam soil (sand: 64%, silt: 25%, clay: 9%) collected from Alam farm Chak no 13/TDA Tehsil Darya Khan 31°47'12″N 71°06'26″E, District Bhakkar, Punjab, Pakistan. The electrical conductivity of soil was 2.0 mS L^{-1} with pH 8.1 having saturation of 32%. Other physicochemical characteristics of soil included organic matter (0.43%), nitrogen (0.044%), potassium (79 mg kg^{-1}), phosphorous 4.6 mg kg^{-1}), chloride (5.4 meq L^{-1}), carbonates (0.4 meq L^{-1}), biocarbonates (6.2 meq L^{-1}), sulphate (6.2 meq L^{-1}), calcium + magnesium (2.1 meq L^{-1}) and sodium (17.9 meq L^{-1}). Iron, copper, zinc, manganese, magnesium and boron found were 4.2, 1.52, 1.24, 3.2, 8.6 and 0.03 mg kg^{-1}, respectively. Soil was sterilized by inserting formalin (2%) soaked cotton plugs at different points, later it was covered with a plastic sheet for 7 days and left unwrapped for another 7 days to assure complete disappearance of formalin fumes. Sterilized soil (4 kg pot^{-1}) was filled in plastic pots (7" × 6" length and width) and was artificially inoculated with freshly prepared inoculum of the fungus prepared on sorghum seed (5 g pot^{-1}). Potted soil was watered and left for one week to establish pathogen inoculum. Ten surface-sterilized seeds of each variety were sown in inoculated potted soil as well as in un-inoculated control treatments. Seven plants were maintained after thinning. Pots were watered after intervals of 24 to 48 hours as per requirement.

The experiment was designed for 60 days, conducted using completely randomized design with three replications of both positive and negative control of each 26 tested varieties.

Disease and growth assessment

Morphological attributes were determined in terms of disease severity in all three replicates of each 26 varieties at 35^{th} and 55^{th} day of sowing. Mung bean plants were regularly examined visually for the disease development by *M. phaseolina* and disease severity was assessed on the basis of disease rating scale (Abawi and Pastor-Corrales, 1990) (Table 1).

Table 1: Disease rating scale (Abawi and Pastor-Corrales, 1990).

Disease rating	Description	Disease reaction
1	No symptoms on plants	Highly resistant
5	Lesions have progressed from cotyledons to about 2 cm of stem tissues	Moderately Resistant
7	Lesions are extensive on stem and branches	Susceptible
9	Most of the stem and growing points are infected. A considerable amount of pycnidia and sclerotia is produced	Highly susceptible

Plant mortality (%) was calculated in each treatment as well. Growth parameters like plant height and weight (fresh and dry weight) were recorded after 60 days and were used to calculate growth inhibition index (GII) to estimate overall reduction in plant growth.

$$GII = \Sigma \frac{SL + SFW + SDW + RL + RFW + RDW}{Total\ number\ of\ perameters}$$

Where: SL =shoot length; SWF = shoot fresh weight; SDW = shoot dry weight; RL = root length; RFW= root fresh weight; RDW = root dry weight

Statistical analysis

Twenty one plants in each treatment of triplicate were individually assessed. Data regarding effect of *M. phaseolina* on plant mortality and growth parameters were subjected to analysis of variance (ANOVA). The differences among means were compared by Fisher's protected least significant difference test (LSD) at level $P \leq 0.05$ by using software Statistics 8.1. Two sample T-Test was applied on growth parameters with respect to their respective control treatments.

Results

The data recorded revealed that all twenty six genotypes of mung bean were differed in their response to charcoal rot disease. These varieties/genotypes were categorized in 4 groups as highly resistant, moderately resistant, susceptible and highly susceptible on the basis of disease severity, mortality and growth inhibition index (GII). Among twenty six genotypes, two were found in highly resistant, ten genotypes exhibited moderately resistant and rest of the fourteen genotypes were found susceptible against effect of *M. phaseolina* (Fig. 1; Table 2).

Two genotypes (MNUYT-317 and NM-2011) were found in highly resistant group exhibited 10% mortality (Table 2). Therefore, the cumulative growth inhibition index of 13-16% in this group was low as compared to rest of three groups (Fig. 1). So far, there was insignificant difference in individual growth parameters of inoculated treatments with respect to their uninoculated treatments (Table 3). The growth inhibition index (%) was ranges between 10-15% over respective control (Fig. 1).

Ten genotypes (Mung-12004, MNUYT-317, Mung-12007, MNUYT-201, MNUYT-219, AZRI-2006, Mung-12002, MNUYT-318, MNUYT-207 and MNUYT-107) were categorized as moderately resistant with disease rating score '5'. In this group, mortality percentage (14-19%) was insignificantly different amongst different varieties/genotype and as compared to highly resistant group (Table 1). Cumulative inhibition in growth (GII) was found within range of 20-30% (Fig. 1). However, different growth parameters and their growth inhibition (%) showed variable response over their respective control treatments (Table 3 and 4).

Seven mung bean genotypes (MNUYT-103, MNUYT-11, MNUYT-301, MNUYT-16 MNUYT-7, MNUYT-109 and MNUYT-312) were grouped in susceptible with disease rating score "7. The mortality (%) was reached up to 30% with GII in the range of 30-40% (Table 1; Fig. 1). Most of the investigated growth parameters were declined significantly in inoculated treatments as compared to their respective control (healthy) (Table 3 and 4).

Rest of the seven germplasms (MNUYT-102, FS-0318, MNUYT-210, MNUYT-118, MNUYT-204, MNUYT-18 and MNUYT-105) acted as highly susceptible (disease rating score "9") to the effect of *M. phaseolina* with the highest mortality (30-60%) (Table 2). Due to high disease severity, the plants showed the maximum reduction in growth parameters with 35-55% GII (Fig. 1). The effect of *M. phaseolina* on individual growth parameters was also significant with the maximum inhibition percentage in comparison to treatments without pathogen inoculation (Table 3 and 4).

Discussion

Charcoal rot disease caused by *M. phaseolina* is a serious threat to mung bean growing areas all over the world and in Pakistan as well that may cause up to 100% yield losses under epidemic conditions (Iqbal *et al*., 2010). Screening of edible crops for their response to disease is suitable and short term process that not only serves as the prerequisite for disease management but also helps in identification of resistant varieties. In current study, twenty six different varieties/genotypes of mung bean were assessed for the difference in their level of resistance against *M. phaseolina* using visual symptoms (disease severity), plant mortality and growth inhibition index. All twenty six varieties/genotypes showed variable response to disease. Generally, disease symptoms were visible in mung bean varieties/genotypes after 27 days of sowing on the collar region of plants. Stem and leaves of the infected seedlings became brown in color. With the passage of time infected green pods turned brownish to black. Mature and dry pods turned into white to gray color, became narrow, deformed and thin after infection. After 35 days, plant started wilted and dried. The same findings were observed by Atiq *et al*. (2014) on mung bean in field artificially inoculated with *M. phaseolina* in Faisalabad, Pakistan.

On the basis of disease ratting scale, two varieties/genotypes were categorized as resistance, ten as moderately resistant and remaining fourteen as susceptible or highly susceptible. Similar differential responses to charcoal rot disease among twenty nine genotypes of mung bean have been observed by Khan and Shuaib (2007). Variations in resistance and low level of resistance amongst twenty seven different mung bean varieties/lines have also been reported previously against charcoal rot disease by Hasseb *at al*. (2013). Likewise, fifty advance lines of mung bean have been assessed against charcoal rot disease with eighteen lines exhibited resistance to moderately resistance response and twenty six were found as susceptible to highly susceptible (Atiq *et al*., 2014).

Current study suggested to evaluate all new germplasm/lines against disease in different agro ecological zones before releasing as approved variety to avoid losses on economic scale. Screening of crops against their response to disease is appropriate technique that besides saving time and labor facilitates its application anywhere and at any time.

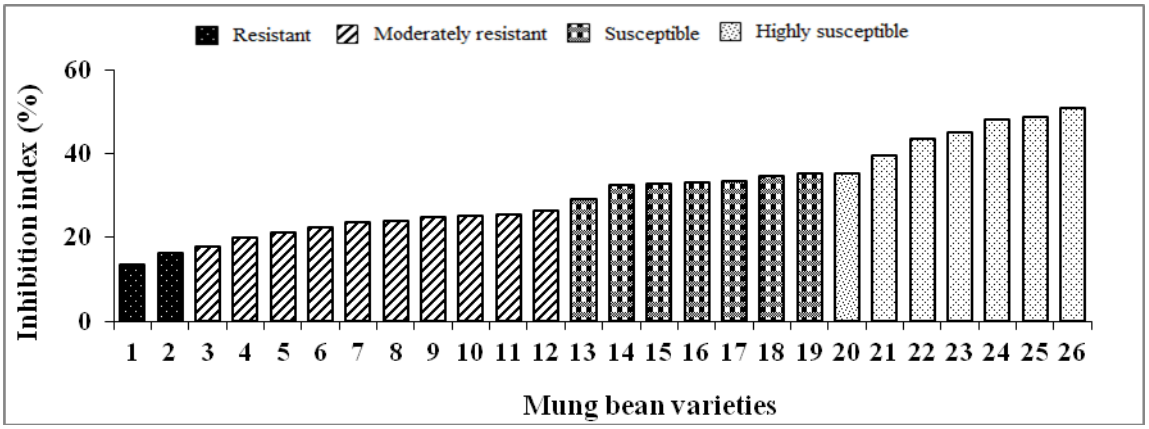

Fig. 1: Growth Inhibition index (%) of different mung bean varieties/genotypes against *Macrophomina phaseolina.*

Table 2: Response of different mung bean varieties/genotypes to *Macrophomina phaseolina* on disease severity and plant mortality.

Response	Mung bean varieties/genotype	Disease severity score scale	Mortality (%)
Highly resistant	MNUYT-317	1	9.33 **g**
	NM-2011	1	9.33 **g**
Moderately resistant	Mung-12004	5	14.00 **fg**
	MNUYT-317	5	19.00 **e-g**
	Mung-12007	5	14.30 **fg**
	MNUYT-201	5	19.30 **e-g**
	MNUYT-219	5	19.00 **e-g**
	AZRI-2006	5	14.00 **fg**
	Mung-12002	5	19.00 **e-g**
	MNUYT-318	5	14.00 **fg**
	MNUYT-207	5	19.00 **e-g**
	MNUYT-107	5	19.00 **e-g**
Susceptible	MNUYT-103	7	28.71 **d-f**
	MNUYT-11	7	29.00 **d-f**
	MNUYT-301	7	24.00 **d-g**
	MNUYT-16	7	28.70 **d-f**
	MNUYT-7	7	23.70 **d-g**
	MNUYT-109	7	29.00 **d-f**
	MNUYT-312	7	33.70 **c-e**
Highly susceptible	MNUYT-102	9	33.70 **c-e**
	FS-0318	9	38.33 **b-d**
	MNUYT-210	9	47.66 **a-c**
	MNUYT-118	9	52.33 **ab**
	MNUYT-204	9	57.00 **a**
	MNUYT-18	9	61.66 **a**
	MNUYT-105	9	61.70 **a**

Letters in a column show significant difference ($P \leq 0.05$) in triplicate mean values as determined by LSD test.

Table 3: Effect of *Macrophomina phaseolina* on growth parameters of different mung bean varieties/genotypes.

Mung bean varieties/Genotype	Shoot growth parameters						Root growth parameters					
	Length (cm)		Fresh weight (g)		Dry weight (g)		Length (cm)		Fresh weight (g)		Dry weight (g)	
	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased	Healthy	Diseased
MNUYT-317	18.44	16.53	3.90	3.42	2.02	1.85	20.89	15.33*	1.08	1.00	0.61	0.51
NM-2011	23.33	19.75	4.93	4.33	2.41	2.00	22.33	19.33	0.98	0.82	0.44	0.34
Mung-12004	20.33	15.42**	5.53	5.15	2.85	2.28*	20.00	17.11*	1.90	1.42	0.87	0.73**
MNUYT-317	20.19	17.95*	4.92	4.86	2.14	1.97	23.22	20.85	2.50	1.27**	1.24	0.74*
Mung-12007	13.44	12.78	1.64	1.57	0.78	0.72*	17.67	15.11**	0.64	0.61	0.24	0.25
MNUYT-201	19.97	13.89*	2.03	1.85	1.22	0.72*	27.00	22.59	0.92	0.79	0.34	0.26
MNUYT-219	23.22	16.18**	3.22	2.72	1.35	1.17	22.89	14.42**	1.75	1.25	0.7	0.59
AZRI-2006	25.00	20.15*	7.71	6.73	3.53	3.40	27.11	23.86*	2.19	1.11*	1.14	0.61*
Mung-12002	26.33	22.78	4.17	2.98	2.13	1.55*	26.33	20.33**	1.83	1.32*	0.83	0.58
MNUYT-318	16.31	12.41**	1.90	1.34	0.85	0.55	22.44	19.44	0.68	0.51	0.29	0.22
MNUYT-207	15.89	14.18*	3.30	2.67	1.49	1.16	23.78	21.41	1.40	0.77*	0.66	0.35*
MNUYT-107	14.78	12.56	1.62	1.14	0.81	0.59	19.00	15.90*	0.73	0.40**	0.29	0.21**
MNUYT-103	18.67	15.40**	2.43	2.02	1.11	0.81*	30.11	25.01*	1.64	0.87*	0.69	0.35*
MNUYT-11	20.00	15.92**	2.70	1.82**	0.72	0.54	23.89	17.84*	1.08	0.64*	0.36	0.17*
MNUYT-301	14.26	11.64	2.02	1.55*	0.87	0.62	20.44	14.64**	0.78	0.43*	0.37	0.17*
MNUYT-16	14.56	10.75**	2.65	1.56*	1.03	0.76*	22.78	16.44*	1.04	0.62**	0.39	0.24*
MNUYT-7	20.78	13.62**	2.26	1.52	1.03	0.55**	27.33	16.56**	1.04	0.91	0.52	0.34
MNUYT-109	25.72	17.22**	7.00	3.42*	2.80	2.07	20.67	17.33	1.59	1.00*	0.8	0.44*
MNUYT-312	15.78	12.85*	4.17	2.75*	2.07	1.08**	23.89	16.7*	1.78	1.13*	0.71	0.39*
MNUYT-102	17.11	13.75**	3.40	1.88*	1.67	0.84*	18.89	16.56**	1.48	0.83*	0.59	0.35*
FS-0318	23.20	17.00*	5.90	2.57*	2.79	1.22*	28.11	22.56*	1.84	1.13**	0.94	0.56**
MNUYT-210	21.63	14.08**	4.87	3.00*	1.58	0.79**	22.78	14.56**	1.06	0.59**	0.56	0.23**
MNUYT-118	21.89	14.29**	5.58	2.92**	2.34	1.42*	25.40	18.00**	2.10	0.86**	1.03	0.40**
MNUYT-204	20.97	13.56**	5.87	1.61*	2.54	0.72**	26.00	17.11**	1.36	0.94**	0.84	0.47*
MNUYT-18	27.00	17.64**	7.92	3.05**	3.10	1.22*	25.44	15.22**	1.99	0.99**	0.91	0.48**
MNUYT-105	21.78	13.97**	8.48	2.25**	3.51	1.12**	28.22	19.52**	0.98	0.47**	0.55	0.31*

* significant at $P \leq 0.05$ and ** significant at $P \leq 0.001$ according to independent Two sample *t* -test comparing healthy and inoculated treatments of each variety/genotype.

Table 4: Inhibition (%) in different growth parameters of different mung bean varieties/genotypes due to effect of *Macrophomina phaseolina*

Disease rating	Mung bean varieties/genotype	Shoot growth parameters			Root growth parameters		
		Length	Dry weight	Fresh weight	Length	Dry weight	Fresh weight
		% inhibition					
Highly resistant	MNUYT-317	10.36 i	12.47 l-n	8.42 h-j	26.60 d-g	28.00 m	15.38 j
	NM-2011	15.36 g-i	12.91 l-n	17.13 g-j	13.43 i-k	16.20 k-m	22.56 h-j
Moderately resistant	Mung-12004	24.18 c-e	6.93 n	20.00 f-j	14.44 i-k	25.26 i-l	16.15 j
	MNUYT-317	11.08 i	11.21 l-n	7.96 i-j	10.20 j-k	49.33 a-c	40.32 b-h
	Mung-12007	17.86 f-h	24.03 h-l	25.86 e-g	26.09 d-g	14.99 lm	18.68 ij
	MNUYT-201	30.44 ab	9.18 mn	41.42c-e	16.33 h-k	49.09 lm	23.30 h-j
	MNUYT-219	30.34 ab	15.54 k-n	13.37g-j	37.01 a-c	28.33 g-k	16.71 ij
	AZRI-2006	19.39 f-h	12.63 l-n	3.59 j	11.99 jk	49.05 a-d	46.63 a-e
	Mung-12002	13.51 hi	28.40 g-k	27.07 e-g	22.78 e-i	28.00 hk	29.44 e-j
	MNUYT-318	23.92 c-f	29.47 g-k	35.43 d-f	13.37 i-k	24.40 j-l	24.42 g-j
	MNUYT-207	10.77 i	19.19 j-n	21.92 f-i	9.96 k	45.26 b-e	46.73 a-e
	MNUYT-107	15.04 g-i	29.98 g-k	26.45 e-g	16.33 h-k	45.52 b-e	26.44 f-j
Susceptible	MNUYT-103	17.48 f-h	16.99 k-n	27.11 e-g	16.94 g-k	46.95 b-e	49.51 a-d
	MNUYT-11	20.38 d-g	32.72 f-j	25.12 e-h	25.33 d-h	40.68 b-f	51.85 a-d
	MNUYT-301	18.39 e-h	23.14 i-m	28.63 e-g	28.40 c-f	44.68 b-e	54.05 a-c
	MNUYT-16	26.79 b-d	41.56 d-g	25.11 e-g	27.11 c-f	41.57 b-g	38.93 c-h
	MNUYT-7	34.44 a	32.99 f-j	46.45 b-d	39.40 ab	31.13 m	34.62 d-i
	MNUYT-109	33.05 a	51.19 b-d	26.28 e-g	16.13 h-k	37.10 d-i	45.42 a-e
	MNUYT-312	18.54 e-h	34.05 e-i	47.90 b-d	30.08 b-e	36.50 e-j	44.34 a-f
Highly susceptible	MNUYT-102	19.64 e-g	44.76 c-f	49.40 b-d	12.35 jk	43.11 b-e	41.57 b-g
	FS-0318	26.71 bc	56.47 bc	56.32 a-c	19.76 f-j	38.51 c-h	40.78 b-h
	MNUYT-210	34.88 a	38.42 d-h	49.89 b-d	36.10 a-c	43.85 b-e	58.08 ab
	MNUYT-118	34.72 a	47.76 b-e	39.37 d-e	29.14 c-f	49.54 a	60.84 a
	MNUYT-204	35.36 a	72.61 a	71.70 a	34.19 a-d	31.72 f-j	44.22 a-f
	MNUYT-18	34.67 a	61.45 ab	60.62 ab	40.17 a	14.18 a-c	46.96 a-e
	MNUYT-105	35.84 a	73.46 a	68.06 a	30.85 a-e	52.54 ab	44.58 a-f

Letters in each row show significant difference ($P \leq 0.05$) in triplicate mean values as determined by LSD test.

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