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Screening of mungbean cultivars under rice allelopathic stress for best agronomic and symbiotic traits

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ASTRACT

In a pot study, effect of 5 and 10% (w/v) aqueous straw extracts of allelopathic rice (*Oryza sativa* L.) variety 'Super Basmati' was investigated on growth, yield, nodulation and arbuscular mycorrhizal (AM) colonization of 8 mungbean [*Vigna radiata* (L.) Wiczek] cultivars (AUM-19, AUM-27, AUM-38, AUM-7375, N-2, N-5, N-8 and N-9). The 10% extract significantly enhanced the shoot and root dry biomass of cv. AUM-27. Contrarily, 5% extract decreased the shoot biomass in cv. N-8 and 10% extract reduced the root biomass in cv. AUM-7375. The 5% extract increased both number and biomass of nodules in cv. AUM-19, while 10% extract decreased them in cv. N-9. The 5 or 10% extract increased the grain yields in AUM-19, AUM-27, AUM-38 and AUM-7375. Conversely, these extracts decreased the grain yields in cv. N-9. The 10% rice extract markedly enhanced the mycorrhizal colonization in N-5, N-8 and N-9 cultivars. The study concluded that for better grain yield under rice allelopathic stress, AUM cultivars of mungbean should be cultivated.

Keywords: Allelopathy, arbuscular mycorrhizae, mungbean cultivars, nodulation, *Oryza sativa*, rice straw extract, *Vigna radiata*

INTRODUCTION

Rice (*Oryza sativa* L.) is the major food crop for more than half of the world population**.** The rice allelopathy (inhibitory or stimulatory influence of one plant on other plants or microorganisms in the environment by exuding chemicals), is well known in rice (16,19,36). Allelochemicals are released into the paddy soil from living rice plants as well as from rice straw left in the fields after harvesting (6,24). The allelochemicals released from living and dead rice plants are substantially different. Momilactone A and B, 3-isopropyl-5-acetoxycyclohexene-2-one-1 and 5,7,4'-trihydroxy- 3",5"-dimethoxyflavone are mainly involved in the allelopathy of living rice plants, while Momilactone B and phenolic acids (*p*-hydroxybenzoic, *p*-coumaric, ferulic, syringic and vanillic acids) resulted from the decomposition of rice straw in paddies are generally involved in allelopathy of rice residues (17,18,20). Rice allelopathy have variable effects on weeds (16,19), crops (9), plant pathogens (2) and beneficial rhizospheric microorganisms (36).

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Mungbean [*Vigna radiata* (L.) Wilczek] is main legume crop in major Asian cropping systems, owing to rich source of protein for human consumption (33). It forms a dual symbiotic association with nitrogen fixing rhizobia (37) and nutrient absorbing arbuscular mycorrhizal (AM) fungi (29). Generally, both rhizobial population and mycorrhizal colonization are adversely affected under allelopathic stress, consequently crop growth and yield are reduced (4,12,13,15). At rice harvest, its residues are incorporated in the soil. The allelochemicals released from such straw materials adversely affect the germination and growth of subsequent crops. Earlier studies have shown that same crop cultivars exhibit differential response to allelopathic stress (14,23). This study aimed to investigate the effects of aqueous shoot extract of allelopathic rice variety Super Basmati on growth, yield, nodulation and AM colonization of 8 varieties of mungbean. This study could help to identify the tolerant cultivar(s) of mungbean for sowing after rice crop.

MATERIALS AND METHODS

In a previous study, we evaluated 254 mungbean genotypes for their best agronomic traits and resistance against mungbean yellow mosaic begomovirus (31). We selected 8 best cultivars *viz*. AUM-19, AUM-27, AUM-38, AUM-7375, N-2, N-5, N-8 and N-9, which have been used in present research. AUM and N stand for Agriculture University Mungbean and NIAB, respectively.

The allelopathic rice variety Basmati Super was selected for this study (9,16). Its straw was collected in November 2008 (after crop harvest) from the field of our University. Rice straw was sun-dried, cut into 1-cm pieces and stored at room temperature. At the time of experiment (February 2009), 10 kg rice straw was soaked in 130 L tap water at room temperature (25±2 °C). The straw was soaked for 8 days and filtered through muslin cloth and about 100 L extract was obtained (10% w/v).

Pot trial

Experiment was conducted in February 2009 in plastic pots (24 x12 cm dia), each containing 2.5 kg soil. The experimental soil was sandy loam [organic matter 0.69%, pH 7.8, EC 1.4 mS cm^{-1}, nitrogen 0.035%, available phosphorus 6.3 mg kg^{-1} and available potassium 100 mg kg^{-1} of soil]. The micronutrients B, Mn, Fe, Cu and Zn were 1.06, 22.8, 10.8, 1.9 and 1.3 mg kg^{-1} of soil, respectively]. The experiment treatments consisted of two factors : (I). Rice shoot extracts 3(0,5,10%) and (II). Mungbean cultivars : 8(AUM-19, AUM-27, AUM-38, AUM-7375, N-2, N-5, N-8 and N-9). The treatments were replicated thrice in completely randomized design. Four seeds of each cultivar of mungbean were sown in each pot and pots were kept in open. The highest and lowest temperatures during the experimental periods were 26.7-35.7 °C and 14.6-22.5 °C, respectively. Relative humidity was 29 to 61%. Bright sunshine duration ranged from 7.15-9.6 h day^{-1}. One week after sowing, thinning was done and two uniform seedlings were kept per pot. Each pot received 1.0 litre of 5% or 10% aqueous extract as per treatment. Distilled water served as control. Two subsequent doses of extracts were applied at weekly interval. All pots were irrigated with tap water whenever required. Plants were harvested 80 days after sowing.

Shoot and root dry biomass, number and fresh biomass of nodules, number of pods per plant, pod length, number of seeds per pod and grain yield per plant were recorded.

Mycorrhizal study

A sub-sample of fresh roots of all the 8 test mungbean cultivars from different treatments were thoroughly washed under tap water and fine roots were cut into 1 cm pieces. The root samples were cleared and stained for AM study following Phillips and Hayman (27). The roots were cleared for about 30 min in 10% KOH solution in an autoclave at 121 °C, placed in 10% HCl for 10 minutes for neutralization and then stained with 0.05% glycerol-trypan blue solution.

Ten stained root pieces from each replicate were randomly selected for AM study. Root pieces were mounted in lactophenol on glass slides and studied under compound microscope. For percentage mycorrhizal colonization, each root piece was observed at 5 points under x10 power of the microscope and % mycorrhizal colonization was calculated. Arbuscular and vesicular colonizations were quantified by counting these structures per cm of root length (15).

Statistical analysis

All the data were analyzed by analysis of variance (ANOVA) followed by Duncan's Multiple Range Test ($P \leq 0.05$) to compare the treatment means (34) using computer software COSTAT.

RESULTS AND DISCUSSION

Vegetative growth

Highest shoot biomass was recorded in mungbean cv. N-8 followed by cv. AUM-19 and cv. AUM-38 and it was markedly higher than other cultivars (Fig. 1). Mungbean cultivars showed variable response to rice shoot extracts. Highest concentration of extract (10%) enhanced the shoot biomass of cultivars AUM-19, AUM-27, AUM-7375 and N-5, However, the stimulation was significant only in cv. AUM-27. Conversely, rice extracts suppressed the shoot biomass of cv. N-8 and cv. AUM-38. The rice extracts slightly influenced the shoot biomass of cv. N-2 and cv. N-9. In general 5% rice shoot extract, suppressed the shoot biomass of all mungbean cultivars except cv. AUM-27.

The 10% rice extracts significantly enhanced the root biomass of cultivars AUM-27, AUM-19, AUM-38 and N-5. In contrast, 10% rice extract significantly reduced (66%) the root biomass of cv. AUM-7375 by 66%. In other mungbean cultivars, the effect of rice extracts was non-significant (Fig. 1).

Nodulation

The 5 and 10% rice extracts increased the number and biomass of nodules in cv. AUM-19. Contrarily, 10% rice extract significantly suppressed both the nodulation and N_2-fixation in cv. N-9. The effect of rice extracts was insignificant in other varieties (Fig. 2). The phenolic compounds and other allelochemicals released from residues of allelopathic plants [*Chenopodium murale*, *Ageratum conyzoides* L. and *Carduus nutans* L]

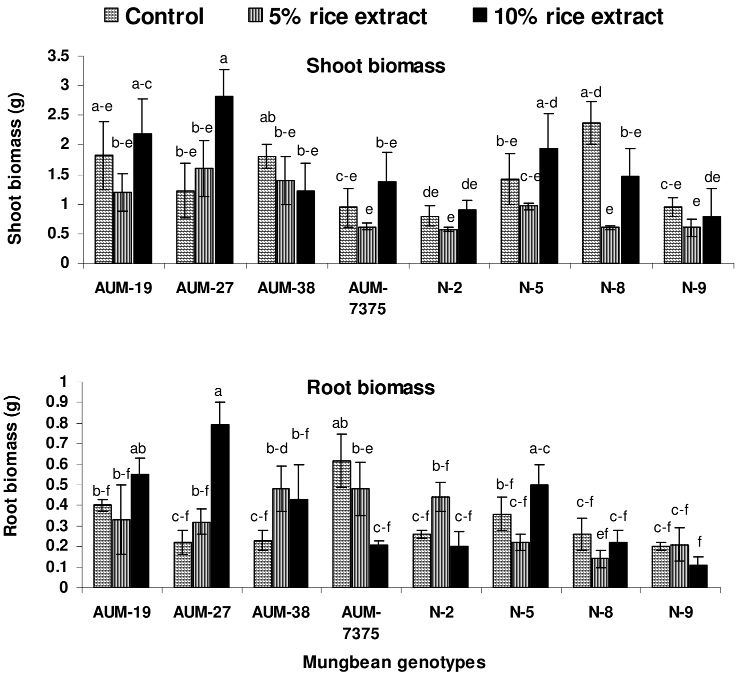

Figure 1. Effects of aqueous shoot extracts of super Basmati rice on shoot and root dry biomass of different mungbean cultivars. Vertical bars show standard errors. Bars with different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

adversely affected the nodulation and N_2-fixation in pea, chickpea and *Trifolium repens* (3,4). Conversely, Heckman and Kluchinski (11) reported that nodule number was increased by 40% for soybean grown in pot soil amended with leaves and crop residues of allelopathic plants at 2 g 100 g^{-1} soil, but nodule dry matter per plant was not influenced than control soil. It seems probable that nodulation response of a leguminous specie varies with the type of allelopathic plant species, amount of residue incorporated or extract added and genotype of the host plant species. Adverse effect of allelochemicals on nodulation generally decreases the nitrogen fixation (3), consequently the crop growth and yield of host plant is also reduced (14). This study revealed that the adverse effects of allelopathic rice straw extract on nodulation of mungbean can be lessened by selecting tolerant mungbean cultivars such as AUM-19.

Yield attributes

In control, mungbean cv. N-8 produced highest number of pods (7.8 per plant) followed by N-9 (5.3 per plant) than other cultivars (1.8-3.2 pods per plant). Application of

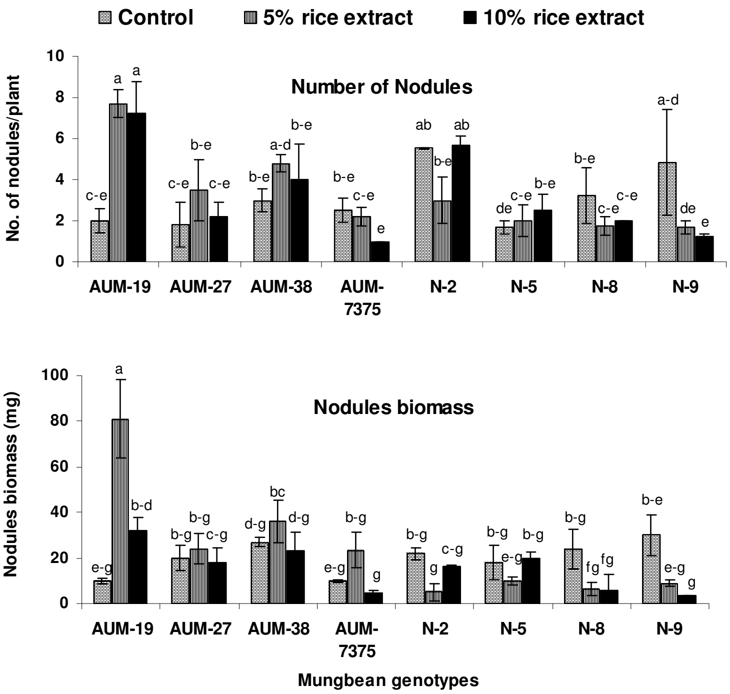

Figure 2. Effects of aqueous shoot extracts of Super Basmati rice on nodulation of different mungbean cultivars. Vertical bars show standard errors of means of three replicates. Bars with different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

10% rice extract markedly enhanced the pod number from 2.5 to 5.7 per plant in cv. AUM-27. Similarly the 5% extracts, increased the pod numbers from 1.8 to 5 and 3.2 to 6 pods per plant in cv. AUM-38 and cv. AUM-7375, respectively. In other cultivars, rice straw extracts did not influence the pod numbers. In general, application of rice extracts did not affect the pod length and number of seeds per pod (Table 1).

In control, mungbean cv N-8 produced highest grain yield (1.7 g plant^{-1}) followed N-2 (0.88 g plant^{-1}). The rice straw extract had variable influence on different cultivars. However, 5% rice straw extract significantly enhanced the grain yield [0.57 to 1.42 g plant^{-1} (149%)] in AUM-38. Similarly 5 and 10% rice straw extracts increased the grain yield in AUM-19 by 78 and 67%, respectively. The 10% rice straw extract caused 128% increase in yield of cv. AUM-27. In cv. AUM-7375, 5% rice extract enhanced the grain yield from 0.53 to 0.85 g plant^{-1} (60%), while 10% extracts caused 53% reduction in yield. While, the rice extracts reduced the grain yield in N-2 and N-9. In other cultivars, effect of rice extracts was not much pronounced (Table 1). These results showed that growth and yield responses of mungbean to allelopathic extracts of rice straw were

Table 1. Effect of different concentrations of aqueous shoot extracts of super Basmati rice on various yield parameters of different mungbean genotypes

Mungbean cultivars	Conc. (%)	No. of pods/plant	Pod length (cm)	No. of seeds/pod	Grain yield (g/plant)	100 grains wt. (g)
AUM 19	0	2.0 de	2.2 c	5.9 a-d	0.46 cd	1.85 c
	5	3.5 b-e	4.1 a-c	6.2 a-d	0.82 b-d	2.32 bc
	10	3.2 c-e	3.6 bc	6.3 a-d	0.77 b-d	2.98 bc
AUM 27	0	2.5 c-e	2.2 c	4.5 cd	0.43 cd	2.04 c
	5	2.0 d-e	2.5 bc	5.0 a-d	0.42 cd	1.98 c
	10	5.7 a-d	3.8 bc	5.2 a-d	0.98 b-d	3.04 bc
AUM 38	0	1.8 e	4.1 a-c	6.5 a-c	0.57 cd	3.87 a-c
	5	5.0 a-e	4.5 a-c	6.2 a-d	1.42 ab	4.45 ab
	10	2.5 c-e	2.3 c	4.8 a-d	0.58 cd	2.36 bc
AUM 7375	0	3.2 c-e	3.3 bc	3.6 d	0.53 cd	5.77 a
	5	6.0 a-c	4.2 a-c	5.9 a-d	0.85 b-d	3.17 bc
	10	3.0 c-e	4.4 a-c	4.7 a-d	0.25 d	3.14 bc
N 2	0	2.2 d-e	2.9 bc	4.6 b-d	0.88 b-d	2.84 bc
	5	3.0 c-e	2.7 bc	5.0 a-d	0.59 cd	3.89 a-c
	10	3.3 c-e	2.4 c	4.3 cd	0.72 b-d	2.34 bc
N 5	0	2.2 d-e	2.7 bc	8.9 a-d	0.65 cd	2.61 bc
	5	3.0 c-e	3.2 bc	5.8 a-d	0.54 cd	1.94 c
	10	3.7	3.5 bc	5.7 a-d	0.72 b-d	3.0 bc
N 8	0	7.8 a	5.1 a-c	7.5 ab	1.7 a	3.29 bc
	5	7.5 a	4.9 a-c	6.3 a-c	1.45 ab	2.36 bc
	10	7.0 ab	6.4 a	7.5 a	1.1 a-c	3.86 a-c
N 9	0	5.3 a-e	3.5 b-c	4.5 cd	0.74 b-d	2.61 bc
	5	5.0 a-e	5.2 ab	6.5 a-c	0.41 cd	3.83 a-c
	10	3.8 b-e	4.0 a-c	6.4 a-d	0.44 cd	1.56 c

In a column, values with different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

genotype dependant. Genotypic variation in growth and yield response of a plant to allelochemicals has also been reported for other plant species (14,23). Toxicity is associated with the presence of strong electrophilic or nucleophilic systems. The action of such systems on specific positions of proteins or enzymes alters their configuration and affects their activity (21).

Mycorrhizal colonization

A great variation in mycorrhizal colonization in different cultivars of mungbean was observed. In control, the 4 cultivars [AUM-19, AUM-27, AUM-38 and N-9] were non-mycorrhizal. The variety AUM-7375 was found highly mycorrhizal (44% colonization) than other cultivars. In N-2, N-5 and N-8 cultivars, there were 7, 21 and 18% mycorrhizal colonization, respectively (Table 2). Variation in mycorrhizal colonization among different genotypes of a species has also been reported in rice, maize, wheat and *Trifolium repens* (8,22,26,28). A marked variation in mycorrhizal response to rice extracts was recorded among the mungbean cultivars. Among the four non-mycorrhizal cultivars, AUM-38 and N-9 became mycorrhizal in response to rice extracts. It reveals that these

Table 2. Effect of different concentrations of aqueous shoot extracts of super Basmati rice on mycorrhizal colonization of different mungbean genotypes

Mungbean genotypes	Concentration (g 100 mL^{-1})	Mycorrhizal colonization (%)	Number of arbuscules/cm	Number of vesicles/cm
AUM-19	0	0 h	0 f	0 d
	5	0 h	0 f	0 d
	10	0 h	0 f	0 d
AUM-27	0	0 h	0 f	0 d
	5	0 h	0 f	0 d
	10	0 h	0 f	0 d
AUM-38	0	0 h	0 f	0 d
	5	27 de	16 a	0.9 cd
	10	0 h	0 f	0 d
AUM-7375	0	44 b-d	9 b-e	3.5 bc
	5	32 c-e	16 a	0.5 cd
	10	42 bc	13 ab	4 b
N-2	0	7 gh	7 c-f	0.3 d
	5	5 gh	1 f	0 d
	10	6 gh	2 f	0 d
N-5	0	21 ef	5 d-f	0 d
	5	13 f-h	5 d-f	0.3 d
	10	63 b	12 a-c	4 b
N-8	0	18 e-g	4 d-f	0.5 d
	5	30 c-e	10 a-d	2 b-d
	10	42 b-d	17 a	2 b-d
N-9	0	0 h	0 f	0 d
	5	11 f-h	3 f	0 d
	10	82 a	15 a	31 a

In a column, values with different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

cultivars were actually weakly mycorrhizal. Generally nutrients availability is reduced under allelopathic stress (1) and these cultivars get benefits from mycorrhizal symbiosis only under stressed conditions. In this study, 10% rice straw extract significantly increased the mycorrhizal colonization and number of arbuscules in cv. N-5 and cv. N-8. In general, number of vesicles was low in all tested mungbean cultivars except cv. N-9, where 31 vesicles/cm of root length were recorded (Table 2). Earlier studies showed variable response of mycorrhizal colonization to allelopathic stress (7). Generally mycorrhizal colonization is suppressed under allelopathic stress (12,13,30,35). However allelochemicals especially phenolic compounds may stimulate the mycorrhizal colonization (32). Depending on the concentration, different phenolic compounds can either increase or decrease the AM fungal spore germination, hyphal growth and hyphal branching (4). In this study, stimulation of mycorrhizal colonization in mungbean cultivars in response to rice extracts may be attributed due to the presence of phenolic compounds in very low concentration (7,25). The increase in mycorrhizal colonization in cultivars N-5, N-8 and N-9 due to rice extracts did not result in a corresponding increase in crop growth and yield. The plant growth response depends on several factors [Effectiveness of the AM host for nutrients assimilation, soil nutrients availability and efficiency of specific host-fungal species association (10)].

CONCLUSIONS

The effects of rice extracts on growth, yield, nodulation and AM colonization in mungbean varied with genotypes and there was no correlation among the different parameters. However, 5 or 10% rice straw extracts increased the grain yield in AUM cultivars, while, the rice extracts reduced the growth and yield in N cultivars,. This study showed that AUM cultivars are more suitable than N cultivars for cultivation under rice allelopathic stress. However, further field studies should be done before giving these recommendations to the farmers.

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