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Response of Mung Beans (Vigna radiate L.) to Agnihotra Homa Ash (AHA) in Terms of Plant Protein and Proline Content

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Abstract

Mung Bean (*Vigna radiata L.*) is an important agricultural plant having wide applications in Ayurveda as well as a rich source of protein for vegetarians. Agnihotra is the basic homa for all homa fire practices and has a positive impact on the environment and living beings around it. To perform Agnihotra homa, cow dung cake, clarified butter and pinch of rice, is used. In the present study, we introduced Agnihotra Homa Ash (AHA) to mung bean plants to evaluate the impact of the AHA on plant protein and Proline content. The estimation was done using 0.25 g and 1.0 g of leaves from the treated and control plants for protein and proline content, respectively. Mung bean plants were grown in pots and further treated with three different treatments viz. 0.50 g, 0.75 g and 1.0 g of AHA. The results indicate that plants treated with 1.0g concentration of AHA have higher concentration of protein and lower concentration of proline (stress indicator). Hence, we can conclude that Agnihotra homa ash not only helps the plant to increase its protein content but also, reduces the stress level of the plant. On the basis of this study, 1g of AHA treatment is suggested for the growth of mung bean plant.

Keywords: Agnihotra Homa; Ayurveda; Homa Ash; Mungbean; Proline; Protein; Sustainable Farming

Introduction

In Asia, most households consume mung beans *(Vigna radiata L.)*, which is grown over 6 million hactare of land (about 8.5% of the worldwide pulse area). In many Asian countries primarily in China, India, Bangladesh, Pakistan, and some Southeast Asian countries mung beans are grown widely because they are not only drought tolerant, and low-input crops, but also have a short growth cycle (70 days or less). Mung beans are a balanced source of protein, dietary fiber, minerals, vitamins, and bioactive compounds. Combining mung beans with cereals has been suggested as a way to improve the quality of protein, since cereals are rich in sulfur-containing amino acids but deficient in lysine while mung has sufficient lysine in it. Mung bean is beneficial for detoxification, to relieve heat stroke, and to regulate digestion. Additionally, it is hypoglycemic, hypolipidemic, antihypertensive, anticancer, anti-melanogenetic, hepatoprotective, and immunomodulatory. Because of the above mentioned properties it is one of the most important crop in India. However, since Mung bean is sown primarily in northern parts of India, it experiences High Temperatures (HT) during its various developmental stages (35-45°C) increasing its stress level, and thereby its prolein level.

The effect of high temperature on mung bean plants includes chlorosis, reduced vegetative and reproductive growth, and abscission of buds, flowers, and pods. Plant's sensitivity to heat stress results in its reduced productivity. Adaptation of physiological and biochemical processes within the plant does lead to improvement in heat tolerance, and at the field level, managing or manipulating cultural practices mitigate some of the adverse effects of HT; also the endogenous levels of plant biomolecules (heat shock proteins, enzymatic and non-enzymatic antioxidants, osmolytes and phytohormones) do shoot up to defend the plant; their expression, however, depends on the type of plant species explored and intensity/duration of the HT. All these defensive measures mentioned above within the plant itself or provided from outside are not able to mitigate entirely the effect of HT on plant growth, hence, alternative options such as the use of AHA certainly needs to be investigated. Agnihotra is a homa farming method to improve nourishment of plants and thereby its yield; AHA also increases the resistance of the crops to harmful radiation and pathogenic bacteria. Various studies have shown that application of AHA on plants has impact on aeromicroflora, decreased air pollution, decreased water pollution and increase in the water extraction of soil phosphates. Reports also indicate that application of AHA on plants enhances seed germination rate, crop producitivity, soil health, and reduces pest problems of plants. Increase in the yield and growth of mushroom fruiting body, increase in the root growth of rice grains and increase in the growth by 40% in Zea mays has been reported when they were exposed to AHA. Application of AHA resulted in increased leaf surface area while exposure to homa vapors showed rapid cell division and fast germination [1].

It would thus appear that AHA may be utilized as an alternate to chemical fertilizers as it is likely not only to increase the growth (yield) of the plant but may also enhance the environmental conditions and may reduce the damage caused by heat stress. Hence, the present study aims to see the impact of AHA on mung bean plant protein content and the Proline content which is a good estimate of the stress level of the plant [2].

Materials and Methods

Agnihotra homa

Agnihotra as a healing fire has its origin in the ancient science of Ayurveda. It is a process of purifying the atmosphere through a specially prepared fire performed at sunrise and sunset daily. Sanskrit chants are

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sung at the same time, which are called mantras. Agnihotra homa is performed in a semi pyramid shaped copper vessel. Dried cow dung cake is placed in the vessel and fire is lit. Unpolished and unbroken rice coated with ghee prepared from indigenous cow is offered while reciting mantras. The homa is performed exactly at sunrise and sunset [3].

The following mantra is chanted

Sunrise Agnihotra mantra

Sooryaya swaha sooryaya idam na mama

Prajapataye swaha prajapataye idam na mama

Sunset Agnihotra mantra

Agnaye swaha Agnaye idam na mama

Prajapataye swaha prajapataye idam na mama

After performing homa, ash is collected to be used for the experiment.

Procurement of seeds of Mung bean

Seeds of Mung beans were procured from the Pulses Section, Gandhi Krishi Vignan Kendra, University of Agricultural Sciences, Bangalore [4].

Experimental set up

Soil mixture was prepared using cow dung, red soil and coco peat in the ratio of 1:1:3 and pots were filled with this soil mixture; total of 40 pots were prepared. In every pot 3 seeds were sown. The AHA solution was prepared by adding 25 gm of AHA to 2.5 liter of water (1 gm AHA/100 ml). The experimental set up consisted of 4 sets of treatments, namely Control (C), 0.5g AHA (T1), 0.75g AHA (T2), 1.0g AHA (T3). The control (C) was not treated with AHA. All the plants were exposed to sunlight to induce heat stress. The plants were irrigated sparingly to avoid complete drying of the soil mixture [5].

Protein Extraction (TCA-Acetone Precipitation Method)

0.25 gram of leaf material was grounded in a precooled mortar in the presence of liquid nitrogen. Approximately 100–150 mg of ground tissue powder was precipitated overnight with freshly prepared 2 mL of 10% Tri Chloride Acetic Acid (TCA) and 0.07% β-mercaptoethanol in cold acetone. Following precipitation the set was centrifuged at 10,000 g for 15-20 min at 4°C and the supernatant discarded. The obtained pellet was rinsed twice in ice-cold acetone with 0.07% β-mercaptoethanol. An additional modification was introduced between the rinsing steps by incubating the sample for 60 min at -20°C. The pellet was air dried, resuspended in 3 ml of sample buffer (8 M Urea, 2% 3-[(3- Cholamidopropyl)Dimethyl Ammonio]-1-Propane Sulfonate (CHAPS), 50 mM Di Thio Threitol (DTT), 0.2% Biolyte 3/10 Ampholyte, 0.001% Bromophenol Blue) (Biorad), and vortexed for 1 hour at room temperature. The supernatant was used for downstream analyses [6].

Protein estimation; Lowry's Method

To the different volumes of supernatant (1.0, 0.8, 0.6, 0.4, 0.2 ml) from above, 2.5 ml of alkaline copper Sulphate reagent was added and thoroughly mixed. Allowed to stand for 10 minutes and then 0.25 ml of Folinis reagent was added. In order to develop colour this was kept standing for 30 minutes. Absorbance was recorded using spectrophotometer at 660 nm, against a blank. The blank was prepared by taking 1.0 ml of 0.5 M NaOH in place of sample in a cuvette. Bovine serum albumin was used to prepare a standard curve and the amount of protein was estimated in different samples [7].

Proline Estimation

Leaf samples were collected from the ten healthy mung plants of each treatment (Table 1). 1 g sample from each treatment was grounded in a mortar after the addition of a small amount of quartz sand and 10 ml of 3% (w/v) aquous sulfosalicylic acid solution. The homogenate was filtered through two layers of glass fiber filter and the clear filtrate was used in the assay. Glacial acetic acid and ninhydrin reagent (1 ml each) were added to I ml of the filtrate (2.5 g ninhydrin/100 mL of a solution containing glacial acetic acid, distilled water and orthophosphoric acid 85% at a ratio of 6:3:1). The closed test tubes with the reaction mixture were kept in a boiling waterbath of room temperature (21°C) for 5 minutes. Readings were taken immediately at a wavelength of 546 nm. The proline concentration was determined from a standard curve [8].

Results and Discussion

Mung bean contains approximately 24 grams of protein per 100g raw weight. Notably, this amount of protein is roughly the same as other animal proteins, making mung beans a perfect choice for vegetarians/vegans. Additionally, the protein in mung beans contains a good range of amino acids in high concentrations. Leaf protein content provides valuable information about physiological status of plants and proline content indicates the stress level of plants. Treating plant soil mixture with different concentrations of AHA, gave different yields of protein content. Results summarized in Table 2 reveal total protein content of leaves at vegetative stage (Day 7). Mean data on protein content revealed that the protein content was highest in T3 compared to control, T1 and T2 (Table 2). Current study further revealed Table 3 that the proline content decreased in treatment T3 compared to control, T1 and T2. So, treatment of plants with AHA at concentration of 1gram, yielded higher quantity of protein and lower amount of proline (stress in plants). We can infer that AHA being an external factor, influences plant growth but there may be an optimum concentration of AHA which is most valuable to plant hence to find that further research is needed [9].

There are many studies available which support the positive impact of agnihotra homa or homa ash, observed the impact of agnihotra homa on germination of rice seeds at day 15 in a petri dish at three distinct seasons (summer, winter and fall). Four other parameters were also considered *viz.* root length, shoot length, fresh weight and dry weight at their vegetative state. The results showed that the germination was more effective than the other four parameters irrespective of seasons when compared with the control. They have noticed the impact of AHA on the solubility of soil Phosphate with three distinct extraction strategies: 48-hour water extraction method, 1-hour water extraction as indicated and Calcium Acetate Lactate (CAL)-extraction as indicated. It was found that AHA may increase the amount of extractable Phosphorus in soil. In another study, the impact of AHA to farm soil was analysed by the microbial count of soil before and after ash addition to the farm soil. The overall increase was observed in bacterial flora, including the

Table 1: Treatments.

Name of treatments	Amount of AHA
C (Control)	NO AHA
T1 (Treatment 1)	0.5 g AHA
T2 (Treatment 2)	0.75 g AHA
T3 (Treatment 3)	1.0 g AHA

Table 2: Effect of different AHA treatments on protein content in Mung bean leaves.

Treatments	Absorbance at 660 nm (R1)	Protein (µg) (R1)	Absorbance at 660 nm (R2)	Protein (µg) (R2)	Protein (µg) (Avg.)
C (Control)	3.001	2014.87	2.999	2013.52	2014
T1 (Treatment 1)	1.533	1029.25	1.534	1029.92	1030
T2 (Treatment 2)	3.351	2249.86	3.353	2251.20	2251
T3 (Treatment 3)	3.727	2502.30	4	2685.60	2594
*R1 (Replicate 1) and R2 (Replicate 2)					

Table 3: Effect of different AHA treatments on proline content in Mung bean leaves.

Treatments	Absorbance at 520nm (R1)	Proline (µg) (R1)	Absorbance at 520nm (R2)	Proline (µg) (R1)	Proline (µg) (Avg.)
C (Control)	0.063	21.09	0.063	21.09	21
T1 (Treatment 1)	0.063	21.09	0.064	21.42	21.2
T2 (Treatment 2)	0.079	26.44	0.078	26.11	26.2
T3 (Treatment 3)	0.034	11.38	0.032	10.71	11
*R1 (Replicate 1) and R2 (Replicate 2)					

effective bacteria i.e nitrogen fixers and phosphate solubilizes while reduction in the fungal flora.

Based on our study and other researchers' observations, it can be said that agnihotra homa farming can be developed as a practice to promote the growth of plants and to reduce the use of agricultural fertilizers. It has the potential to act as fertilizer as well as to improve the health and quality of the soil by maintaining concentration of micronutrients present in it, and thus, promoting the development of sustainable agriculture as indicated in the Vedic scriptures [10].

Conclusion

On the basis of results obtained by us and earlier reports, Agnihotra farming or Homa farming can be considered as scientific and safe way of organic farming. The technique helps in increasing the plant protein content and it decreasing the stress level of the plant as indicated by decreased proline content; thus providing a sustainable and safe option for farming. There is a need to strengthen this neglected area of vaidic farming by conducting more research in this area.

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Conflict of Interest

None

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