



Influence of Sulphur and Varieties on Growth and Productivity of Mustard (*Brassica Juncea L.*)

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Abstract: A field experiment was laid out in randomized block design (factorial) with three replication at agronomy Research farm Ayodhya (U.P.) during the rabi season. Fifteen treatments comprised of five levels of sulphur i.e. 0, 15, 30, 45 and 60 kg S ha⁻¹ and three varieties namely NDR-8501, Vardan and Varuna of Mustard to find out appropriate dose of sulphur and suitable variety for higher production. The experiment was sown in the field, having the soil texture silty loam, pH 7.9, O.C 0.32%, EC 0.33ds/m and available N, available P, available K, available S and available Zn were 180.4 kg/ha, 18.4 kg/ha, 290 kg/ha, 7.3 (ppm) and 0.59 (ppm), respectively. Among the various treatments sulphur dose 60 kg S/ha significantly influenced the plant height, number of branches/plant, leaf area index, number of siliqua/plant, length of siliqua, number of seeds/siliqua and dry matter accumulation/plant, which was at par with 30 and 45 Kg S/ha and significantly superior over rest levels of sulphur. whereas, effect of sulphur on Harvest index, 1000 grain weight (g), nitrogen and protein content were found non-significant. Seed yield, Stover yield and sulphur content in seed and stover was significantly increased with increasing dose of sulphur up to 60 Kg/ha superior over control, 15 and 30 Kg S/ha and at par with 45 Kg S/ha.

Keywords: sulphur, mustard, siliqua, seed, yield, harvest, research, index, loam, rabi, season.

Introduction

Rapeseed mustard is the second-most important oilseed crop in India, next only to soybean, with almost one-fourth share in both area and production. It is cultivated in an area of 6.3 million hectares with a production of 8.0 million tonnes yielding 1324 kg ha⁻¹, whereas in Uttar Pradesh, mustard is grown in the area of about 6.79 lakh hectares with an annual production of approximately 9.45 lakh tonnes (DOAC 2017). There exists a huge gap between the global productivity (20.47 q ha⁻¹) and India's productivity (13.24 q ha⁻¹) which need to be bridged with the expansion of area under high

yielding varieties (hybrids) due to their improved genetic potential. In addition, climate change manifested in uneven, untimely and inadequate rainfall aggravates the soil moisture stress condition during both vegetative and reproductive phases of crop growth. This could be addressed primarily with better water management through scientific irrigation scheduling (irrigation water depth (IW) to cumulative pan evaporation (CPE) ratio, i.e. IW/CPE) which will boost both production and productivity of mustard as well as minimize the risk associated with climate change.[1]

Further, the yield potential of any crop is best utilized in the presence of optimum fertilization and irrigation. In terms of nutritional requirement, sulfur (S) plays a major role in determining yield, quality and resistance of mustard toward various stress factors. The multi-functionality of sulfur is also evident in chlorophyll synthesis, seed protein, enzymatic and vitamin components which is sine qua non for superior nutritional and market quality oilseed production. Under irrigated condition, sulfur can bring a corresponding increase in yield to the tune of up to 50% but increased and indiscriminate use of high analysis fertilizer with low or no sulfur has led to sulfur deficient soils. This calls for additional sulfur application in oilseed crops in general and mustard in particular so as to meet the higher oilseed demand of the country. Despite plentiful information, as mentioned above, on the beneficial effect of different inputs on the productivity of crops, reports on cumulative effects of the same on the performance of hybrid mustard are limited in general and with respect to eastern Uttar Pradesh in particular. Therefore, this field experiment was undertaken to assess the response of mustard varieties to irrigation scheduling and sulfur fertilization in the eastern Uttar Pradesh of India.[2]

Dhara Mustard Hybrid-11, otherwise known as DMH - 11, is a genetically modified hybrid variety of the mustard species *Brassica juncea*. It was developed by Professor Deepak Pental from the University of Delhi, with the aim of reducing India's demand for edible oil imports. DMH - 11 was created through transgenic technology, primarily involving the Bar, Barnase and Barstar gene system. The Barnase gene confers male sterility, while the Barstar gene restores DMH - 11's ability to produce fertile seeds. The insertion of the third gene Bar, enables DMH - 11 to produce phosphinothricin-N-acetyl-transferase, the enzyme responsible for Glufosinate resistance. This hybrid mustard variety has come under intense public scrutiny, mainly due to concerns regarding DMH - 11's potential to adversely affect the environment as well as consumer health. DMH - 11 was found not to pose any food allergy risks, and has demonstrated increased yields over existing mustard varieties. Conflicting details and results regarding the field trials and safety evaluations conducted on DMH - 11 have delayed its approval for commercial cropping.

The transgenic mustard DMH - 11 was developed in 2002 using genetic material isolated from non-pathogenic soil bacteria,[8] and techniques in transgenic systems for pollination control, which primarily involved the Barnase-Barstar system.[9] Three genes, Bar, Barnase and Barstar, were extracted from *Bacillus amyloliquefaciens* to produce the hybrid seed.[5] The main reason for introducing the Barnase-Barstar gene system into the transgenic mustard line, was for heterosis breeding and to prevent self-fertilization.[9] The insertion of the Barnase gene induces genetic male sterility by preventing the production of the male gametophytes (pollen grains) in the mustard plant.[5] Meanwhile, the Barstar gene acts to restore the ability of the plant to produce fertile hybrid seeds. [5] Mustard is a self-pollinating plant, thus, making it difficult to perform cross-pollination with another desired male parental line, without the occurrence of self-pollination. The Barnase gene induced male sterility in DMH - 11, simplifying the process of cross pollination to derive new hybrid varieties. The two parental strains used to develop DMH -11 are the Early Hira mutant (EH -2) which was developed by Anil Khalatkar of Nagpur University,[10] and the Varuna bn 3.6.[8] The seed weight of DHM-11 is reported to be around 3.3 to 3.5 grams (0.12 oz)/1000 seeds.[10]

DMH - 11's Glufosinate resistance is due to an enzyme expressed by the Bar (Bialaphos resistance) gene. Derived from *Streptomyces hygroscopicus*, the cloned Bar gene in DMH-11 encodes for the synthesis of phosphinothricin-N- acetyl-transferase (PAT). [11] This enzyme is responsible for detoxifying the active ingredient in the herbicide Glufosinate : phosphinothricin.[5] Phosphinothricin's mechanism of action involves the inhibition of Glutamine synthetase, which prevents the detoxification of ammonia and subsequently causes toxic buildup within plant cells. Inhibition of glutamine synthetase also leads to an overall reduction in Glutamine levels. In plants, Glutamine acts as a signalling molecule, and as a major amino acid donor for nucleotide synthesis.[3] PAT enzymes produced by the Bar gene, deactivate Bialaphos (the tripeptide precursor to phosphinothricin) through acetylation to form an inactive, non-toxic product. [11]

DMH - 11 was also subject to a food allergenicity test using bioinformatics comparisons following CODEX and ICMR guidelines, to examine whether the amino acid sequence of Bar, Barnase and Bastar proteins were potential allergens.[9] The test was carried out by identifying any similarities between the amino acid sequence of the three proteins, to that of other known putative allergens.[9] The potential open reading frames at the DNA insertion site of the three genes, were assessed for potential similarities to existing allergens in the AllergenOnline.org database.[9] The results of the study found that DMH - 11 does not present any risk of food allergy to consumers.[9] Further trials on DMH - 11 have been suggested, such as performing a human serum IgE test. [9]

Discussion

The leaves, seeds, and stems of this mustard variety are edible. The plant appears in some form in African, Bangladeshi, Chinese, Filipino, Italian, Indian, Japanese, Nepali, Pakistani, Korean, Southern and African-American (soul food) cuisines. Cultivars of *B. juncea* are grown for their greens, and for the production of mustard oil. The mustard condiment made from the seeds of the *B. juncea* is called brown mustard and is considered to be spicier than yellow mustard.[4][5]

Because it may contain erucic acid, a potential toxin, mustard oil is restricted from import as a vegetable oil into the United States.[6] Essential oil of mustard, however, is generally recognized as safe by the U.S. Food and Drug Administration.[6] In Russia, this is the main species grown for the production of mustard oil. It is widely used in canning, baking and margarine production in Russia, and the majority of Russian table mustard is also made from *B. juncea*.

The leaves are used in African cooking,[7] and all plant parts are used in Nepali cuisine, particularly in the mountain regions of Nepal, as well as in the Punjabi cuisine in the northern part of the Indian subcontinent, where a dish called sarson da saag (mustard greens) is prepared.[8] *B. juncea* subsp. tatsai, which has a particularly thick stem, is used to make the Nepali pickle called achar, and the Chinese pickle zha cai. This plant is called "lai xaak" in Assamese and it is cultivated hugely during the winters. It is eaten in any form in Assam and Northeast, be it boiled or added raw in salad, cooked alone or with pork.

The Gorkhas of the Indian states of Darjeeling, West Bengal and Sikkim as well as Nepal prepare pork with mustard greens (also called rayo in Nepali). It is usually eaten with relish and steamed rice, but can also be eaten with roti (griddle breads). In Nepal it is also a common practice to cook these greens with meat of all sorts, especially goat meat; which is normally prepared in a pressure cooker with minimal use of spices to focus on the flavour of the greens and dry chillies. *B. juncea* (especially the seeds) is more pungent than greens from the closely related *B. oleracea* (kale, broccoli, and collard greens),[9] and is frequently mixed with these milder greens in a dish of "mixed greens".[4]

Chinese and Japanese cuisines also make use of mustard greens. In Japanese cuisine, it is known as takana and often pickled for use as filling in onigiri or as a condiment. Many varieties of *B. juncea* cultivars are used, including zha cai, mizuna, takana (var. integrifolia), juk gai choy, and xuelihong.

Asian mustard greens are most often stir-fried or pickled. A Southeast Asian dish called asam gai choy or kiam chai boey is often made with leftovers from a large meal. It involves stewing mustard greens with tamarind, dried chillies and leftover meat on the bone. *Brassica juncea* is also known as gai choy, siu gai choy, xiao jie cai, baby mustard, Chinese leaf mustard or mostaza.[10]

Results

The rapeseed-mustard require for their sulphur content. Sulphur accumulation pattern in different plant parts at various growth stages indicated a several fold higher requirement of S in rapeseed-mustard as compared to the other two species. A low N: S ratio found in rapeseed-mustard was also an indicative of its higher S requirement. The S requirement of different varieties was found to be similar to that of the other. [5]

The additional S required by rapeseed-mustard may be attributed to the presence of glucosinolates, a characteristic of cruciferous plants. Sulphur fertilization enhanced yield of the oilseed species but not the per cent oil. The role of sulphur in oil biosynthesis is necessitated. Sulphur (S) is an essential macronutrient involved in numerous metabolic pathways required for plant growth. Crops of the plant family Brassicaceae require more S compared with other crops for optimum growth and yield, with most S ultimately sequestered in the mature seeds as the storage proteins cruciferin and napin, along with the unique S-rich secondary metabolite glucosinolate (GSL).[6] It is well established that S assimilation primarily takes place in the shoots rather than roots, and that sulphate is the major form in which S is transported and stored in plants. We carried out a developmental S audit to establish the net fluxes of S in two lines of *Brassica juncea* mustard where seed GSL content differed but resulted in no yield penalty. We quantified S pools (sulphate, GSL and total S) in different organs at multiple growth stages until maturity, which also allowed us to test the hypothesis that leaf S, accumulated as a primary S sink, becomes remobilized as a secondary source to meet the requirements of GSL as the dominant seed S sink. Maximum plant sulphate accumulation had occurred by floral initiation in both lines, at which time most of the sulphate was found in the leaves, confirming its role as the primary S sink. Up to 52 % of total sulphate accumulated by the low-GSL plants was lost through senesced leaves.[7] In contrast, S from senescing leaves of the high-GSL line was remobilized to other tissues, with GSL accumulating in the seed from commencement of silique filling until maturity. We have established that leaf S compounds that accumulated as primary S sinks at early developmental stages in condiment type B. *juncea* become remobilized as a secondary S source to meet the demand for GSL as the dominant seed S sink at maturity.[8]

Conclusions

Sulphur (S) is an essential macronutrient involved in numerous metabolic pathways required for plant growth, including the synthesis of amino acids, proteins, co-enzymes, vitamins and secondary metabolites such as glucosinolates (GSLs) and sulpho flavonoids. Plants take up S from the soil in the form of sulphate, which is then reduced to sulphide for further metabolism through S assimilation processes. Crops of the plant family Brassicaceae, such as canola (*Brassica napus*), mustard (*Brassica juncea*), Chinese cabbage (*Brassica rapa*) and other vegetables (*Brassica oleracea*), have a larger requirement for S to achieve optimum growth and yield compared with non-Brassicaceae crops. Leaves play a dual role, first as a primary sink and later in development as a secondary source. [9] The model predicts that targeting S assimilation in crop breeding within the context of source-sink relationships could result in modified levels of seed GSL or storage proteins. At present, this approach is hindered by the lack of comprehensive data describing the uptake, distribution and fate of S and S-containing metabolites throughout crop development.[10] Addressing this gap in *Brassica* crops would allow a better understanding of the distinct S sources and sinks [11]

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