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Water Stress Induced Changes in Seed Quality of Fenugreek

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Abstract: Seed quality of fenugreek is determined by its primary metabolites and an alkaloid diosgenin. Apart from these fenugreek seeds contains total oil, saponins, carbohydrates, protein and major nutrients. Seeds are bitter in taste due to presence of an alkaloid "trigonelline". In India, major cultivated area of fenugreek is still dependent on conserved moisture and moisture stress conditions reduces its productivity.

Keywords: water stress, seed quality, fenugreek, productivity, laboratory, metabolites, nutrients, oil, diosgenine.

Introduction

A field experiment was carried out in randomized block design (RBD) with three replications during *Rabi* 2016-2017. Performance of ten fenugreek genotypes was evaluated on the basis of seed quality parameters under normal and water stress condition. Seed samples from both normal and water stressed plant were used for quality analysis in laboratory. Analysis of different quality parameters was done by using standard methods and protocol.[1]

Water stress was found to increase seed total oil, saponins and diosgenine content in all the genotypes. Genotypes RMt-1, RMt-305 and RMt-143 were able to accumulate significantly higher total oil and diosgenine content in normal as well as water stress conditions. Water stress condition induced more saponins synthesis in genotype RMt-361 and RMt-351 while crude protein and total carbohydrate declined in all the genotypes. The minimum decrease in crude protein and carbohydrate due to water stress was recorded in genotype RMt-351 and genotypes[2] Hisar Sonali respectively. Moisture was found to decrease in all the genotypes under influence of water stress conditions, minimum (13.75%) being observed in genotype RMt-305. Maximum seed yield was recorded in genotype RMt-361 both under normal as well as water stress conditions. Genotype RMt-1 had least reduction[3] in yield under water stress condition (3.54%). Results suggested that a significant genotypic variation in seed quality parameters of fenugreek genotypes induced through stress may be utilize to introduce novelty of different purpose for developing new cultivars.[4]

Total oil (%) Twenty gram seeds of fenugreek genotypes were ground and oil was extracted with 150.0 ml of n-hexane following the procedure of Soxhlet (1879) extraction. Total Oil (%) = [{(Weight of oil + Weight of flask) – (Weight of flask)}/Weight of sample] 100 Saponin content (%) The defatted cake (residue after oil extraction) was kept under the hood overnight. The next day 100 ml methanol was added to the cake and the mixture was shacked overnight on a rotary shaker at 50 rpm. This mixture was centrifuged at 4000 rpm for 5 minutes. [5]The centrifugation process was repeated three times and supernatant were pooled. Methanol was evaporated in a hood to obtained yellow crystal powder of crude sapogenins. Diosgenin content (%) Diosgenin was determined as per the methods with some modification. Standard Diosgenine and p-Anisaldehyde were procured from sigma-aldrich, USA. All other chemicals were of analytical grade[6]. The diosgenin level was determined by measuring absorbance at 430 nm, based on the colour reaction with anisaldehyde, sulphuric acid and ethyl acetate. Briefly, two colour developing reagent solutions were prepared: (A) 0.5 ml p-Anisaldehyde (99%) and 99.5 ml ethyl acetate and (B) 50 ml concentrated sulphuric acid and 50 ml ethyl acetate. A 1000 ppm solution of crude saponins was prepared in methanol. Hundred milli litre of this solution was placed in another glass tube. The methanol was evaporated under reduced pressure[7]. This residue was dissolved in 2 ml of ethyl acetate; 1 ml each of reagents A and B were added to the tube and stirred. The test tube was C for 10 minutes to°placed in a water bath maintained at 60 develop colour and then allowed to cool down for 10 min in C. The absorbance of the developed colour was°25 measured with a spectrophotometer (Systronics, type no 2203) at 430 nm. Ethyl acetate was used as a control for the measurement of absorbance.[8] As a reagent blank, 2 ml ethyl acetate was placed in a tube and assayed in similar manner. For the calibration curve, 10-80 ppm standard diosgenin in 2 ml ethyl acetate was used. Each sample was repeated thrice and the average was taken. The amount of diosgenin was calculated by using the standard curve of diosgenin prepared with methanol. Moisture percent Moisture content was determined using oven dry method. Crude protein (%) Percent nitrogen was estimated by digesting the samples with sulphuric acid using hydrogen peroxide to remove black colour. Estimation of nitrogen was done by colorimetric method using Nessle` s reagent to develop colour. [9] Protein content in grains was calculated by multiplying nitrogen content (%) by the factor 6.25. Total Soluble Sugar (%) Hundred milligram of the fenugreek seed powder was extracted with 5 ml of boiling 80% ethanol. Solution was centrifuged at 5000 rpm for 10 min using a table top centrifuge machine. Supernatant was collected in a small beaker and evaporate it on a hot plate using fume hood.[10] The residue was dissolved in 10 ml dH2O. Pipette out 0.2 ml of the sample extract and make up the volume to 1.0 ml with dH2O. Four ml of anthrone reagent was added in each test tube slowly and mix carefully. Test tubes were place inboiling water bath for 10.0 min. Take out the test tubes and let them come to room temperature. Optical density (O. D.) of blue green colour of cooled sample was recorded at 620 ηm. Quantity of carbohydrate was expressed as mg/g of the sample[11]. Ash content (%) Ash Content was determined as described in the AOAC (1995) and calculated as percent according to below formula. Ash = (Wt. of ash/ wt. of sample) 100 Test weight (g) The sample of 1000 grain was taken from each plot and their weight was recorded using an electronic balance. Seed yield per plant For seed yield five plants were selected randomly of each genotypes and yield was calculated in seed yield per plant (g). Statistical analysis All the observation was taken in each genotypes, replications and sets. The data was statistically analysed[12].

Discussion

Fenugreek crop responds to water stress in the form of changes in various physiological, biochemical and molecular processes. Research on water stress tolerance mechanisms in fenugreek has gained momentum in many laboratories around the world. In the present study, with ten genotypes varying in quality performance in response to limited irrigation, quality indices have been monitored at two environment conditions, i.e., normal irrigation and limited irrigation. Biochemical parameters were measured for quality evaluation[13] (viz., Total oil, Saponin, Diosgenin, Crude protein, Total soluble sugar, Moisture percent, Ash content. Beside these yield parameters were also measured i.e. test weight and seed yield per plant. [14]All these parameters helped in assessing the quality of fenugreek genotypes under water stress condition. The values for total oil in the fenugreek genotypes revealed that there was increase in total oil percent under moisture stress in all genotypes[40]. The total oil varied from 2.68% (RMt-305) to 3.95% (RMt-1) under non stress conditions, while under moisture stress it varied from 3.43% (RMt-351) to 4.34% (RMt-1). [15]Thus, study showed significant increase in total oil content due to water stress. The maximum increase was observed in genotype RMt-305 (33.42%) followed by RMt-351 (22.62%) and RMt-303 (15.05%) Effect of moisture deficiency on oil content in seeds has been studied also by earlier researchers. General observation is that seed oil reduced in most of the oil seed crops when moisture stress imposed at later stage of plant development. However, contrasting observations were made[39]. They reported non-significant effect on seed oil of groundnut varieties on account of moisture deficiency at either early or late growth stage.[16] Similarly, it was also reported no effect of irrigation regimes on seed oil content. In present investigation, we found slightly increased seed oil content when moisture stress applied and provide normal irrigation. They reported that water stress at mid term growth stage, increased the oil content from a minimum of 3.29% to maximum of 5.31%. In our study, water stress observed increase in total oil content in all the genotypes. Water stress at different growth stages of plant might alter the seed composition and related quality. [17] The saponin content varied from 4.68% (RMt-305) to 5.59% (RMt-361) under non stress, while under limited moisture stress it varied from 5.08% (RMt-305) to 5.80% (RMt-361). The maximum increase was observed in genotype RMt-351 (18.64%) followed by RMt-143 (14.11%) and RMt-305 (8.61%). The results showed significant increase in saponins content due to limited moisture stress [38]. Fenugreek is a rich source of steroids such as yamogenine, tigogenin and diosgenin. Diosgenin is the most impartment out of all four mentioned secondary metabolites. [18]Total saponin and diosgenin content in seeds of fenugreek genotypes grown under water stress are presented. The Diosgenin content varied from 0.62% (RMt-354) to 0.84% (RMt-1) under non stress, while under water stress it varied from 0.66% (RMt-354) to 0.91% (RMt-1). Thus the present investigation showed significant increase in Diosgenin content due to limited irrigation. The maximum increase due to limited moisture stress was observed in genotype RMt-143 (14.71%) followed by Hisar Sonali (10.23%) and Rajendra Kanti (9.76%). On the basis of increase in Diosgenin under moisture stress, genotype RMt143 seems to be tolerant to water stress. Several researchers evaluated fenugreek germplasm for sapogenin and diosgenin content and reported 0.28-0.92% diosgenin [19]and 0.92-1.68% steroidal saponins (Arivalagan et al. 2013) in mature seeds of fenugreek which is similar to present report. In the present study the moisture percent was found to decrease, in all the genotypes during water stress[37]. The values of moisture percent varied from 5.51% (RMt-354) to 5.93% (RMt-365) under non stress control, while under water stress it varied from 4.42% (Rajendra Kanti) to 4.79% (RMt361). The minimum decrease due to water stress was observed in genotype RMt-305 (13.75%) followed by RMt143 (16.26%) and RMt-354 (17.26%). Results of present investigation showed significant reduction in seed moisture content under water stress condition. The moisture percent in the seeds is of utmost importance, if the moisture % is higher to a certain level it may creak the fungus effect on the seed[36], while on storage i.e. lower the percent better for quality point of view[20]. Genotype Rajendra Kanti has lowest moisture % in limited moisture stress while higher reduction in moisture % was noticed in genotype RMt-365 (23.33%). Seed crude protein varied from 23.75% (RMt-365) to 27.56% (Hisar Sonali) under non stress control, which decreased slightly when water stress was applied to 22.94% (RMt-365) to 26.98 % (Hisar Sonali). The minimum decrease was, however, observed in genotype RMt-351 (1.44%) followed by RMt-1 (1.53%) and Hisar Sonali (2.10%). The fenugreek seeds were the richest source of crude protein. Researchers have reported that fenugreek seeds are rich in protein[35] with a well-balanced amino acid pattern. This could probably be attributed to the increase in N2-fixing efficiency of inoculated plants where more nitrogen was fixed and translocated to the seed. There are reports indicating decrease in crude protein content in seeds of lentil under water stress.[21]

Results

The total soluble sugar was also found to reduce under moisture stress. It was recorded 4.44% (Rajendra Kanti) to 5.86% (RMt-365) under non stress and reduced up to 3.80% (RMt-351) to 5.66% (Hisar Sonali) under water stress conditions[34]. Minimum decrease was observed in genotypes Hisar Sonali (1.62%) followed by RMt-361 (3.47%) and RMt365 (4.21%)[22]. Ash content is the digestion of mineral matter had significant variation among genotypes. Water stress was, however, found to be reduced in all the genotypes. The ash percent varied from 3.54% (RMt-303) to 4.59% (RMt-351) under non stress conditions, while under moisture stress it varied from 3.10% (RMt-1) to 4.29% (RMt-354). Amongst the ten genotypes, the lowest reduction was observed in genotypes RMt-354 (1.05%) followed by RMt-143 (2.63%) and RMt-303 (3.09%) due to water stress [23].The presence of ash in fenugreek plant in such quantities are satisfying, because of the high importance of mineral for health maintenance and development. Ash consider basic element of biomolecules (protein, enzyme, phospholipid) in addition to their roles in connectivity process and in all of biochemical reaction (Jim, and Stewart, 2002). Our results are consistent with the findings in lentil crop [24]. Test weight (g) ranged from a minimum of 11.01g (Rajendra Kanti) to the maximum of 14.44 g (RMt-361) under non stress control while under water stress it reduced and ranged from a minimum of 10.69g (Rajendra Kanti) to the maximum of 13.94g (RMt-361). All the genotypes showed significant reduction in test weight under moisture stress conditions. However, minimum decrease was observed in genotype RMt-365 (2.65%) followed by Rajendra Kanti (2.92%) and RMt-303 (3.05%). [33]The findings are in consonance and it was found Test weight of seeds is directly proportional to seed moisture content. In present investigation seed moisture content was also found to reduce under water stress conditions resulted in less test weight. A significant reduction in seed yield was recorded in response to water stress irrespective of the genotypes. It was ranged from 2.78 g per plant[25] (Rajendra Kanti) to 4.56 g per plant (RMt-361) under stress as compared to 3.92 g per plant (Rajendra Kanti) to 5.67g per plant (RMt-361) under non stress conditions. Genotype RMt-1 had least reduction in yield under water stress condition (3.54%) followed by RMt-365 (10.34%) and RMt-303 (17.29%) with a maximum reduction in genotype Rajendra Kanti (29.19%) indicating that this genotype is most susceptible to moisture stress conditions. In an earlier report, it was found varietal difference in drought resistance of fenugreek. The highest phenotypic and genotypic variances are found in seed yield[26] under rain fed conditions and plant type and growth habit in both rains fed and irrigated conditions.It is reported that the missing of 4th irrigation in fenugreek decreased fresh and dry weight by 52.96 and 47.3% as compared with control plants. Thus it can be concluded that, an extensive water stress tolerance mechanism[32] is active in fenugreek crop. Drought susceptibility of a genotype is often measured as a function of the reduction in yield under drought stress. In the present study, there is reduction in yield due to water stress in all the genotypes studied. Genotype RMt-1 was less affected in limited irrigation. [27]

Conclusions

Underutilized crops like fenugreek should be seen as an alternative for crop diversification in the areas of limited water availability[31] for satisfying nutritional requirement of increasing population. The results of present investigations indicated significant effect of water stress on quality parameters where an increase was observed in total oil, saponin content[28] and diosgenin content while decreases in crude protein, TSS content, moisture percent, ash content, mineral composition, test weight and seed yield. Genotype RMt-1 accumulates high total oil and diosgenine content while the genotype RMt1 accumulates high total oil and diosgenine content under both the conditions. Hisar sonali was least affected for crude protein and total soluble sugar under stress conditions. Study revealed that fenugreek can be grown with limited moisture by judicious[29] application of available water without affecting quality attributes viz., total oil, diosgenin content and saponin content; rather, it increases under moisture stress. Fenugreek genotypes like RMt-361 and RMt-1, are rich in steroidal saponin, diosgenin, could be utilized as alternative sources for the synthesis of steroid drugs in pharmaceutical industries.[30]

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