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Comparative Gas chromatography-Mass spectrometry (GC-MS) analysis of two cultivars of *Hordeum vulgare* L.

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Abstract

Hordeum vulgare L. is a multi-nutritional food grain crop of the Poaceae family. It is a significant staple crop in Asia with significant nutritional and commercial importance. It is possible to grow this plant under a number of abiotic conditions of stress, such as fluoride stress. The objective of the current study was to determine whether two chosen cultivars of *Hordeum vulgare* could endure or tolerate fluoride stress conditions by using gas chromatography-mass spectrometry (GC-MS) analysis. The two *H. vulgare* cultivars' seeds that were chosen, RD2786 and RD2794, were planted in pH-5 soil and allowed to mature for a month in clay pots. Different concentrations of fluoride (0, 5, 10, 15, 20 ppm) were added to the soil as supplements. Moreover, GC-MS was used to assess factors such as protein content and nitric oxide. Additional TLC analysis was carried out using a TLC sheet. As a result of free radical production, the cultivar RD2786 had higher total protein and nitric oxide scavenging enzyme (NOSA) activity than RD2794. Because of its osmoprotectant properties and ability to tolerate fluoride stress conditions, cultivar RD2786 of *H. vulgare* is therefore advised for farmers in fluoride-affected regions based on the results of this study.

Keywords

Hordeum vulgare L.; Fluoride stress; GC-MS; Poaceae; Abiotic; Nitric oxide

Introduction

Primarily, *Hordeum vulgare* L., or barley, is a cereal grain that is being grown around the world due to its affordability and nutritional worth. After rice, wheat, and maize, the fourth-most popular crop in the world to be cultivated is barley (1). In western countries, this crop is mostly used as feed, though it can also be used in small amounts for food and malt. Worldwide, barley is a staple crop that is used everywhere (2). When compared to other grasses, barley has the highest concentration of minerals and nutrients, even at the beginning of its growth. According to Lee and Nishiyama (3,4), barley is also helpful as a natural remedy for a number of illnesses, including anaemia, cancer, renal problems, high cholesterol, circulatory diseases, obesity, and diabetes.

Due to the large amount of dietary fibre in barley, especially soluble glucan, which has been shown to lower insulin and postprandial serum glucose and its response linked to impaired glucose tolerance, there has been a significant shift in focus in barley utilization recently (5, 6). The reduction of postprandial blood glucose and insulin response linked to decreased glucose tolerance has been demonstrated by arabinoxylan, which is also gaining popularity (7, 8). Its endosperm's cell wall is easily broken, giving it a greater potential for resistance in maize and wheat, which lowers the amount of nutrients in the digestive system. Due to its two key qualities-

high nutritional content and low glycemic index—barley is becoming more and more popular worldwide (9, 10).

Fluoride (F^-) is a halogen anion and the thirteenth most prevalent element in the crust of the earth. Coal, soil, clay, and rocks all contain fluorides, namely sodium fluoride. In addition, mineral weathering and volcanic ash emissions, especially marine aerosols, release them into the atmosphere. Soil readily absorbs the extremely reactive halogen fluoride, which combines with a variety of cations to create complexes. Phosphate fertilizer usage in agriculture and atmospheric F^- particles both contribute to soil fluoride pollution (11). In water with low pH and hardness, inorganic fluorides are frequently found. F is not a required element for plants, but its presence in the air, water, or soil can alter plant biochemical, physiological, and structural processes in an unfavourable way, which can have a number of detrimental repercussions (12). Soil fluoride pollution is caused by both excessive use of phosphate-based fertilizers in agriculture and particulate matter from the atmosphere. Stevens et al. (1997) state that F^- toxicity in plants causes foliar damage and impacts physiological processes like respiration and photosynthesis in addition to enzyme activity (13, 14). Research has demonstrated that barley displays dangerous signs at even lower F^- concentrations (15). F^- has an impact on plant growth and development by interfering with numerous metabolic processes involved in protein and nucleotide synthesis (16, 17).

Materials and methods

The seeds were obtained from Krishi Vigyan Kendra (Banasthali Vidyapith, India) to test the growth of two barley cultivars (RD2786 and RD2794) under various fluoride (as NaF) concentrations. The selected cultivars' seeds were sterilized using 0.01 percent $HgCl_2$, and they were subsequently washed with autoclaved water. After four days, the seedlings from the sterilized seeds were transferred to pots in petri plates. Three seedlings were transplanted in duplicate into each 18-litre pot. Ten kilograms of soil with a pH of 7.3 at room temperature were added to each pot, and the soil was then mixed with 25% ammonia solution by volume to bring the pH down to 5.2. The pH, soil, and water solution were measured using a glass electrode in a 1:2:5 ratio (18). The light brown colour, sandy texture, and 0.12 kg^{-1} organic content of the soil were observed. Wet digestion was used to determine the organic content of the soil (19). For a month, the pots were kept in a culture chamber at a temperature of 27 °C. On the fourth, eighth and twelfth days, treatments were given, and the control plants were given distilled water. In plants that were 30 days old, the accumulation of F^- was examined at different F concentrations (0, 5, 10, 15, and 20 PPM).

Using BSA as the standard, the total protein content was calculated using Lowry's method (22). The reaction mixture (3 ml) containing extract or standard solution (0.5 ml), phosphate buffer saline (0.5 ml), and sodium nitroprusside (10 mM, 2 ml) was incubated at 25 °C for 150 minutes in order to estimate NOSA. Following incubation, 1 ml of sulfanilic acid reagent (0.33% in 20% glacial acetic acid) was pipetted into 0.5 ml of the reaction mixture containing nitrite. The combination was then left to stand for 5 minutes to complete the diazotization process. Subsequently, 1 milliliter of naphthyl ethylene diamine dihydrochloride was introduced, combined, and left to stand at 25 °C for 30 minutes. When light is dispersed, a mophore forms. At 540 nm, the absorbance of these solutions was determined. As per Badami et al. (23), the amino acids on the chromatograms were identified using the ninhydrin-collidine chromogenic reagent for TLC amino acid detection following two-dimensional development. According to Jones and Heathcote (25), the reagent was sprayed over the thin layers, which were subsequently kept in a stream of heated air.

Extraction process

Fresh leaves were removed, thoroughly cleaned, and allowed to dry for 7 days at room temperature before being crushed with liquid nitrogen (20). A control sample of both cultivars and 1 g of each leaf powder with varying concentrations of F^- were placed in a thimble and extracted for 12 hours with methanol using a soxhlet device. When the temperature dropped below 35 °C, the extracts were concentrated using a rotatory flash evaporator at a reduced pressure. Following that, the protein content and secondary metabolites of the extracts were identified and evaluated using established techniques (21).

Result

Quantitative determination of total protein content

Protein content was recorded higher at control (leaves, seeds) compared to other treatments, for both the cultivars (Table 1.). Among the treated plants, which received treatment protein content was increased in better cultivar RD2786 in leaves and seeds (8.84±0.04 mg g^{-1} FW, 22.74±0.01 mg g^{-1} FW), respectively. While, treated cultivar RD2794, showed lowest protein content in leaves and seeds at 20 ppm (6.41±0.01 mg g^{-1} FW, 18.93±0.06 mg g^{-1} FW), respectively.

Nitric oxide scavenging enzymes (NOSA)

NO has the potential to produce hydroxyl radicals and nitric oxide. *H. vulgare* cultivars RD2794 and RD2786 NOSA were shown to have antioxidant activity of 51.09±0.04 and 55.08±0.08 (µg/ml; Mean ± S.D, n=3), respectively, at 20 ppm (Table 2). Lower antioxidant activity is indicated by a higher IC_{50} value. According to Sang et al. 2008 (24), the sources of NO synthesis under water stress, the part NO plays in hydrogen peroxide (H_2O_2) accumulation caused by water stress, and the subcellular activities of antioxidant enzymes in maize (*Zea mays* L.) plant leaves were all examined. In response to water stress, nitric oxide synthase (NOS) activity is increased in the cytosolic and microsomal fractions of maize leaves, while nitric oxide (NO) synthesis increases in maize mesophyll cells. Water stress-induced defensive increases in NO production were

Table 1. Protein content in two cultivars, viz., RD2786 and RD2794 of 120 days old *Hordeum vulgare* L. leaves and seeds after fluoride stress

	Total protein content (mg gfw-^{1})			
	RD2786		RD2794	
	LEAVES	SEEDS	LEAVES	SEEDS
Control	8.84±0.04^{c}	22.74±0.08^{c}	6.41± 0.01^{c}	19.52±0.06^{c}
5 ppm	6.12± 0.07^{c}	20.05±0.06^{c}	4.34±0.01^{b}	18.93±0.06^{c}
10 ppm	5.72± 0.02^{b}	18.14±0.05^{b}	3.89±0.05^{b}	14.51±0.08^{b}
15 ppm	4.62±0.02^{a}	16.11±0.01^{b}	2.24±0.01^{a}	13.76±0.03^{a}
20 ppm	3.43±0.01^{a}	14.19±0.01^{a}	2.03±0.01^{a}	10.03±0.01^{a}

Data are the same means and standard deviation of the mean for n = 3 independent experiments.

Table 2. Nitric Oxide Scavenging Activity of *Hordeum vulgare* L. cultivars under F- (0, 5, 10, 15, 20 ppm)

(F) ppm	RD2794	RD2786
Control	36.28±0.07^{a}	38.28±0.06^{a}
5 ppm	39.39±0.03^{ab}	40.20±0.09^{b}
10 ppm	41.25±0.03^{b}	42.25±0.05^{bc}
15 ppm	44.29±0.06^{c}	47.86±0.01^{c}
20 ppm	51.09±0.04^{d}	55.08±0.08^{d}

For n=3 independent experiments, the data are the same means and standard deviations.
a , b and shows the presence of homozygosity in results ($p < 0.001$).

inhibited by pretreatments with inhibitors of NOS and nitrate reductase (NR), suggesting that NO is produced in the leaves of maize plants exposed to water stress via NOS and NR.

Thin layer chromatography

The production of distinctively coloured spots facilitated the identification of various amino acids, such as tryptophan, leucine, histidine, lysine, cysteine, proline, valine, phenyl alanine, methionine, and arginine (Fig. 1), easier. Since the actual size of the spots determines the effective separation of adjacent spots, the quality of any

Fig. 1. Shows the actual sizes of the spots and the effective separations produced by thin layer chromatography.

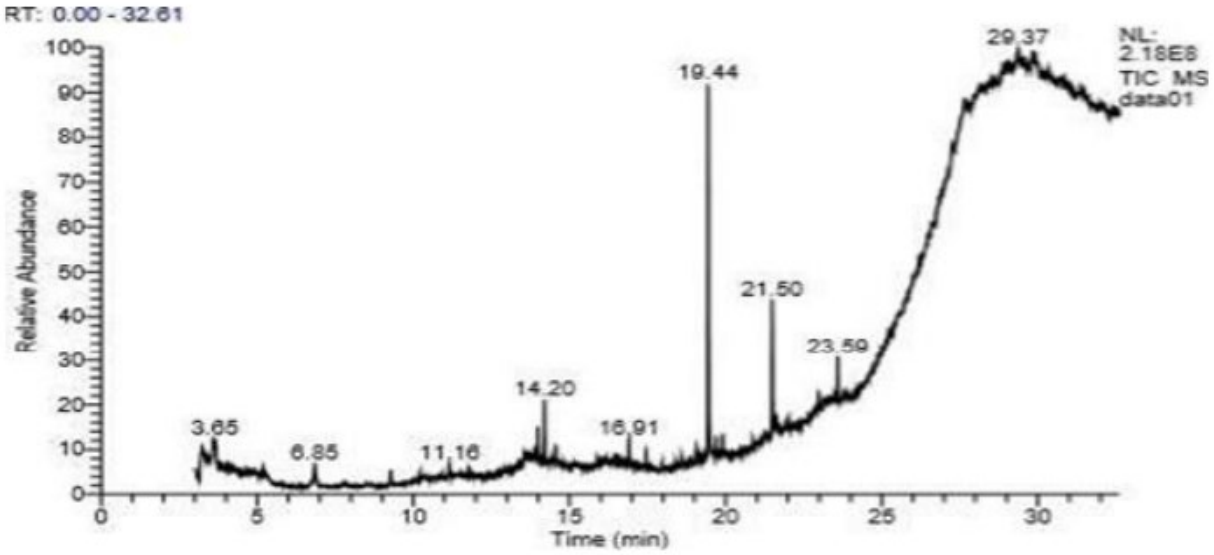

Fig. 2. Determination of bioactive compounds through GC-MS.

separation cannot be evaluated only based on knowledge of the RF values of the spots.

Determination of bioactive compounds through GC-MS

The leaves were extracted in methanol and analyzed on a Thermo Scientific Triple quadrupole GC-MS using a TG 5MS (30m, 0.25mm, 0.25m) column (trace 1300 GC, Tsq 8000 tripe quadrupole MS). Helium gas was used as a carrier gas with a flow rate of 1ml/min and a volume of 1.0L. The injector temperature was kept at 250 °C, the oven temperature at 500 °C, and the ion source temperature was kept at 230 °C (26). The RD2786 barley in methanol extract is subjected to GC-MS analysis, and the results show the presence of pharmacologically active compounds (Table 3.) with anti-inflammatory, antioxidant and antifouling, characteristics. The development of novel plant-based medicines for the treatment of a range of

Table 3. GC-MS analysis of *H. vulgare* L. cultivars of RD2786 in methanol extract

S/No	RT	Area	Phytochemicals	Class of Phytochemicals	Molecular Formula
			Boric acid	Monobasic Lewis acid	H_3BO_3
1	3.25	7.38%	Trimethyl Phosphoric acid	Trialkyl phosphates	$(C_2H_5)3PO_4$
			[methyl(formyl)amino] methyl(2phenylethy)	Benzene	C_6H_6
2	3.64	6.68%	Trimethylsilyl (TMS) derivatives	Ester	$C_{13}H_{23}NSi$
3	13.99	4.19 %	Heptacosane	Alkanes	$C_{17}H_{36}$
			Octadecane	Alkanes	$C_{18}H_{38}$
4	19.44	37.23 %	Hexadecanoic acid	Fatty acid	$C_{16}H_{32}O_2$
			Methyl ester	Mono-alkyl esters	C_2H_3O2R
5	21.50	13.75 %	Methyl stearate	Fatty acid methyl esters	$C_{19}H_{38}O_2$
			Heptadecanoic acid	Fatty acid	$C_{17}H_{34}O_2$
6	23.60	4.62%	Dodecanoic acid	Medium-chain fatty acids	$C_{12}H_{24}O_2$
7	27.64	6.75%	DI-n-octyl phthalate	Benzoic acid esters	$C_8H_8O_2$
8	29.85	8.75 %	Rhodopin	Protein	$C_{40}H_{58}O$

diseases may therefore get benefit from the use of the extract of cultivar RD2786 of barley.

Discussion

The greater quantities of protein and nitric oxide found may scavenge free radicals created during fluoride stress. Secondary metabolite identification in response to fluoride treatment protects the plant from oxidative stress while also increasing its bioactive potential and therapeutic capabilities. A GC-MS study revealed nine distinct compounds in the methanol extract of the barley cultivar known as RD 2786. In addition to functioning as an antioxidant, hexadecanoic acid; methyl ester (RT 19.4) possesses nematicide, hypocholesterolemic, and pesticide properties. These are just a few of the chemicals identified by GC-MS that are known to have numerous significant formulation and biological roles (27). As an alkane with antibacterial and anti-algal properties, hexacosane is alkanes (28). According to Wu et al. 2018 (29), methyl stearate regulates the porosity of the cell wall, offers resistance against toxicity, and guards against abiotic stress. Dodecanoic acid is a saturated fatty acid with antifungal effects (27). 1, 4-Diol-2-methylbenzene has inflammatory properties. Di-n-octyl phthalate is a plasticizer with antibacterial and antifouling qualities (29). Higher plants are thought to have rhodopsin-like retinal-binding protein(s) based on the observation that they have all-trans-retinal (30). Because both cultivars were grown under the same environmental conditions and stresses, the differences are due to their genetic makeup. The considerable variances found in this study are crucial for getting the optimum utilization of these barleys as food and feed.

Conclusion

According to our research, protein content is found to be high in RD2786 and lower in RD2794. This suggests that RD2786 is a desired crop to cultivate under fluoride stress. Throughout the research, the fluoride-tolerant and susceptible types are identified based on a number of essential parameters. The results of the suggested study project might also aid in the development of plant breeders' resistance to fluoride stress in barley.

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Authors' contributions

This work was carried out in collaboration among all authors. AA has conceptualized the topic. AS collected all available literature and wrote the first draft of the manuscript. CB critically reviewed the study. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: "None".

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