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Foliar γ-Aminobutyric Acid Alleviates Heat-Induced Growth Inhibition and Modulates Physiological and Biochemical Responses in Fine Fescue (*Festuca rubra* L.)

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Abstract

Heat stress severely limits the growth and physiological performance of cool-season turfgrasses, including fine fescue (*Festuca rubra* L.). γ-Aminobutyric acid (GABA) is an important signaling metabolite involved in osmotic adjustment and antioxidant defense, but its role in heat-stressed fine fescue remains poorly characterized. This study investigated the effects of foliar-applied GABA on the morphological, physiological, biochemical, antioxidant, and mineral responses of fine fescue exposed to heat stress. Plants were grown under optimum (23/18 °C day/night) or heat-stress conditions (43/37 °C day/night) and foliar-sprayed with 0, 0.5, 1-, or 2-mM GABA in a completely randomized factorial experiment with four replicates. Heat stress markedly reduced plant growth, relative water content, photosynthetic pigments, and leaf N, P, K, and Ca concentrations, while increasing electrolyte leakage (EL), malondialdehyde (MDA), and superoxide dismutase (SOD) activity by 4.6-fold, 6-fold, and 56.5%, respectively, relative to optimum temperature. GABA application, particularly at 1 mM, alleviated heat-induced injury by improving leaf water status, maintaining chlorophyll and carotenoid contents, increasing soluble sugars and proline, and enhancing antioxidant defense. At 1 mM GABA, SOD activity increased by approximately 40% compared with the untreated control under heat stress and was accompanied by lower EL and MDA accumulation. Although GABA partially improved mineral uptake under heat stress, differences among GABA concentrations were not significant for N, K, and Ca. Overall, foliar GABA application improved the physiological and biochemical adjustment of fine fescue to heat stress, with 1 mM providing the most consistent protective response.

Keywords: Antioxidant enzyme activity; Cool-season turfgrass; Electrolyte leakage; *Festuca rubra* L.; Mineral nutrient status; Relative water content

Introduction

Heat stress is a major environmental factor limiting plant growth and development[1,2]. It occurs when temperatures exceed a critical threshold for a sufficient period to impair normal plant growth and development, with the physiological effects depending partly on the duration of exposure[2,3]. The severity of this stress depends not only on the absolute temperature but also on the duration of the stress and the rate of temperature increase[4,5]. Cool-season grasses are

particularly sensitive to heat stress because their optimal growth typically occurs at relatively low temperatures (18 to 23 °C) whereas summer conditions can expose them to temperatures far exceeding their physiological optimum (30 to 35 °C daytime/ 23 to 28 °C nighttime)[6]. Consequently, rising temperatures and the increased frequency of extreme heat periods associated with climate change pose a growing challenge for maintaining the growth and quality of cool-season grasses in landscape and urban environments[7,8,9].

Fine fescues (*Festuca* spp.) are important cool-season turfgrasses widely used as lawns, golf courses, and other green spaces due to their fine leaf texture[10,11], and ability to grow in soils with relatively low fertility and acidity, as well as in moderate shade[11,12]. However, their quality typically declines during the summer a condition often caused by heat stress, drought, or a combination of both, known as summer decline[11,13]. Notably, heat stress has been reported to be more detrimental to fine fescues than drought stress, highlighting the importance of heat tolerance for maintaining their performance throughout the summer[11]. There is also significant variation in heat tolerance among different fine fescue taxa and cultivars[8]. Compared to some other fine fescue taxa, hard fescue has generally exhibited superior heat tolerance, whereas Chewings fescue, strong creeping red fescue, and slender creeping red fescue have shown varied responses to high temperatures [11, 14]. These variations suggest that the heat response in fine fescues is a complex that may be determined by the genetic background and physiological status of plants.

High temperatures can induce a wide range of morphological, physiological, and biochemical disorders in plants[5]. Direct effects of high temperatures include protein denaturation, alterations in membrane lipid fluidity, and disruption of cellular structures, while prolonged heat exposure can impair enzyme activity, protein synthesis, photosynthesis, and membrane function[15]. In cool-season turfgrasses, this damage typically manifests as reduced vegetative growth and turf quality, decreased chlorophyll, diminished photochemical efficiency, and compromised membrane stability[16,17].

One significant consequence of heat stress is the disruption of cellular redox balance. High temperatures trigger the generation of reactive oxygen species (ROS)[5,18], and the excessive accumulation of these compounds can damage membrane lipids and reduction of membrane semi-permeability, plant pigments[19], decreases protein synthesis and promotes protein degradation[20], and injure other cellular components. Consequently, lipid peroxidation and membrane instability are key outcomes of heat stress in cool-season turfgrasses, closely associated with increased EL and the accumulation of MDA[16,17]. In response to this oxidative stress, plants activate antioxidant systems involving enzymes such as superoxide dismutase

(SOD), catalase (CAT), peroxidase (POD), and ascorbate peroxidase (APX)[18,19,21], which play a vital role in controlling or scavenging ROS. Thus, the ability to maintain antioxidant capacity and membrane stability is considered a crucial component of heat tolerance in cool-season turfgrasses[22].

Heat stress also interrupts plant water relations[23], and nutrient uptake[24], both of which are crucial for plant growth and physiological function under high temperatures. A reduction in leaf relative water content (RWC) is a common response in turfgrasses exposed to heat stress and reveals a disruption in the plant water status during heat stress[16,17,25]. Furthermore, high temperatures can negatively alter nutrient uptake and tissue concentrations by inhibiting root mass and root architecture[26], as well as the decrease in nutrient uptake proteins[24] required for nutrient absorption and translocation. Thus, maintaining adequate water status and nutrient balance is a vital component of the plant response for survival and constant growth under heat stress.

Among the compounds studied for amplifying plant tolerance to abiotic stresses, gamma-aminobutyric acid (GABA) has garnered substantial attention due to its diverse metabolic and signaling roles. GABA is a non-proteinogenic, four-carbon amino acid widely distributed in various organisms[27]; it typically accumulates rapidly in response to environmental stressors, such as high temperatures[28,29]. This compound is primarily synthesized from glutamate via the enzyme glutamate decarboxylase and enters the GABA shunt within mitochondria, regulating carbon and nitrogen metabolism[27]. GABA shunt is a pathway linking GABA metabolism to the provision of carbon skeletons and energy for downstream biosynthetic pathways[30]. Beyond its metabolic function, GABA is involved in cytosolic pH regulation, osmoregulation, carbon-nitrogen balance, ROS homeostasis, and stress-response signaling[29,31]. This function offers a solid physiological basis for evaluating GABA as a potential osmolyte for improving plant tolerance to abiotic stressors.

Evidence from research on turfgrass showed that exogenous GABA can boosts the physiological function of creeping bentgrass under heat stress. Foliar application of GABA mitigated heat-induced growth inhibition and reduced water loss, while maintaining higher RWC, improving cell membrane stability, reducing oxidative damage, and improving photosynthetic activity[32], chlorophyll retention[33], and stomatal conductance[21]. GABA-treated plants also exhibited lower EL, MDA accumulation, and carbonyl compound content, alongside higher antioxidant capacity and increased activities of the SOD, CAT, POD, and APX enzymes under heat stress conditions[21]. In creeping bentgrass, exogenous application of GABA has been reported to affect the status of several mineral nutrients under heat stress

conditions; So that an increase in N, P, Ca, Na and Cu and a decrease in B and Mn were observed[25]. These findings indicate that GABA-induced heat tolerance may be related to changes in mineral nutrition in addition to water relations, osmotic regulation, antioxidant defense, and carbon metabolism.

Despite these findings, evidence regarding GABA-induced heat tolerance in turfgrasses stems primarily from studies on creeping bentgrass, while data concerning fine fescues remains limited [21,25,28,32,33]. This is significant because, as previously noted, different fine fescue taxa exhibit noticeable differences in their response to high temperatures[11,34], and the physiological traits associated with heat tolerance. Furthermore, the degree of protection conferred by GABA can depend on the species, stress intensity, stress duration, and GABA concentration. Therefore, the current study was conducted with the aim of investigating the effect of foliar application of GABA on growth, physiological and biochemical responses, antioxidant defense and nutrition absorption of fescue (*Festuca rubra* L.) under optimal temperature and heat stress conditions. In this study, plant growth, RWC, photosynthetic pigments, EL, MDA, soluble sugars, proline, SOD activity, and the concentration of N, P, K, and Ca elements in leaves were considered to determine the effect of GABA on water status, membrane integrity, osmotic regulation, oxidative protection, and plant nutritional status under heat stress conditions.

Materials and methods

Plant material, growth conditions, and experimental treatments

In this experiment, 0.7 g of seeds of fine-leaved fescue (*Festuca rubra* L.) were sown directly in plastic pots with 15 cm high and 15 cm in diameter. Pots were filled with a mixture of sand, farm soil, and composted cow manure at a ratio of 2:1:1. The soil utilized had a sandy-loamy texture with an acidity level of 7.31, an electrical conductivity of 1.2 dS m^{-1}, and an organic matter content of 3.53%. After one month of establishment in the greenhouse, plants were maintained under natural sunlight conditions and ambient greenhouse temperature, and were irrigated uniformly every three days. The experimental treatments consisted of two temperature regimes [optimum temperature (23 °C day/18 °C night) and heat stress (43 °C day/37 °C night)] and four GABA concentrations (0, 0.5, 1, and 2 mM) with four replications. After one month of establishment in the greenhouse, the first GABA foliar application was performed, followed by a second application seven days later. Thus, the total greenhouse establishment period was 37 days. GABA was sprayed uniformly onto the foliage of the plants, and distilled water in an

equal volume was applied to the control treatment. After the second foliar application, plants were transferred to a growth chamber maintained initially at 23/18 °C (day/night), 380 μmol m^{-2} s^{-1} PAR, 50% relative humidity, and a 12-h photoperiod. During the first two days, the temperature was gradually increased in the growth chamber until the target temperature for each treatment was reached, with plants assigned to the heat treatment reaching 43 °C during the day and 37 °C at night. This gradual temperature increase was implemented to prevent shock from sudden temperature change. Heat stress was then maintained at the target temperature for seven days, after which a third GABA foliar application was performed. Final sampling for trait measurement was conducted 14 days after the third foliar application.

The temperature and humidity of the greenhouse were controlled by a digital thermometer and hygrometer (LX8011). Light measurements were recorded by a light meter (L732013, Lutron, Chinese Taipei). During the experiment, the average light intensity was approximately 10,300 lux, and the average temperature was 25 ± 1 °C. Additionally, the relative humidity was approximately 54%.

Turf quality and plant growth measurements

Turfgrass VQ was assessed using a 1–9 visual rating scale based on overall color, density, and uniformity, following Beard[35] method. A score of 9 represented excellent quality (a dense, uniform, dark-green turf), whereas 1 indicated completely dead turf. Visual quality was initially standardized across treatments to minimize pre-existing variation among experimental units. At the end of the experimental period, shoots were separated from the crown and weighed immediately to determine fresh shoot weight (SFW). The samples were then placed in paper bags and oven-dried at 70 °C for 48 h, or until a constant weight was reached. After cooling to room temperature, the samples were weighed to determine shoot dry weight (SDW). Roots were subsequently removed from the growth medium and gently washed with water to remove any adhering substrate. Excess surface moisture was removed with paper towels, and root fresh weight (RFW) was recorded immediately. Root samples were then oven-dried at 70 °C for 72 h to constant weight, cooled to room temperature, and weighed to determine root dry weight (RDW).

Photosynthetic pigments, leaf water status, and membrane stability

To determine the total chlorophyll and carotenoid contents of the leaves, the method described by Lichtenthaler[36] was employed. Briefly, 1.0 g of fresh leaf tissue was weighed and thoroughly homogenized in a porcelain mortar containing 80% acetone. Following complete extraction of the pigments, the extract volume was adjusted to 10 mL with 80% acetone. The extracts were then centrifuged at 8,000 rpm for 10 min. After centrifugation, the supernatant was used for the determination of photosynthetic pigments, and absorbance was measured using a spectrophotometer at 663, 645, and 470 nm. Pigment concentrations were calculated using the following formula 13 and are expressed as mg g^{-1} FW:

Chlorophyll a:12.25(A663.2) − 2.79(A646.8)

Chlorophyll b:21.21(A646.8) − 5.1(A663.2)

Total chlorophyll: Chlorophyllb + Chlorophylla

Total carotenoids: (1000×A470) − (1.82×Chla) − (85.02×Chlb)/198

Electrolyte leakage (EL) was determined according to Sullivan and Ross[37]. Briefly, 0.1g of fresh leaf tissue was cut into approximately 1-cm segments and rinsed three to four times with distilled water to remove surface-adhering electrolytes. The tissue segments were transferred to glass tubes containing 15 mL of distilled water and incubated at 25 °C for 24 h. The samples were gently agitated for 15 min, and the initial electrical conductivity (EC1) was recorded using a conductivity meter. The samples were then autoclaved at 0.1 MPa for 15 min to release the total electrolytes from the tissues. After cooling to 25 °C, the final electrical conductivity (EC2) was recorded. The relative EL became calculable as initial EC (%) over final EC.

Relative water content (RWC) was determined according to the method described by Sairam et al.[38]. Briefly, 2.0 g of fresh leaf samples were immersed in 50 mL of distilled water and maintained at room temperature for 4 h to achieve full turgidity. The turgid weight of the leaves was then recorded using a digital balance. Subsequently, the samples were oven-dried at 70 °C for 48 h to determine their dry weight. Relative water content was calculated using the following equation:

$$RWC\ (\%) = [(FW - DW) / (TW - DW)] \times 100$$

where FW is fresh weight, DW is dry weight, and TW is turgid weight.

Proline and MDA determination

Leaf proline content was determined according to the method described by Bates et al.[39]. Briefly, 2 mL of the prepared alcoholic extract was transferred into capped 15-mL test tubes, followed by the addition of 2 mL of acid ninhydrin reagent and 2 mL of glacial acetic acid. The resulting mixture was incubated in a boiling-water bath for 1 h and then immediately transferred to an ice bath for rapid cooling and termination of the reaction between the amino acids and ninhydrin. After cooling, 4 mL of toluene was added to each test tube, and the tubes were vigorously vortexed for 15–20s. This procedure facilitated the separation of the organic and aqueous phases and resulted in the formation of a colored compound in the upper, toluene-containing phase. The absorbance of the upper phase was measured immediately at 520 nm using a spectrophotometer.

Malondialdehyde (MDA) content was determined using the thiobarbituric acid (TBA) assay described by Heath and Packer[40], with modifications. Briefly, 3 g of fresh leaf tissue was homogenized in 15 mL of 5% (w/v) trichloroacetic acid (TCA) and centrifuged to obtain a clear supernatant. An aliquot (1 mL) of the supernatant was mixed with 2 mL of the TBA reaction reagent. The mixture was heated in a water bath, cooled in an ice bath, and centrifuged to remove precipitated material. Absorbance was measured at 532 and 600 nm. The absorbance at 600 nm was subtracted from that at 532 nm to correct for nonspecific turbidity. MDA content was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1}, with appropriate corrections for extraction volume, reaction dilution, and sample fresh weight, and expressed as μmol g^{-1} fresh weight.

MDA= (OD532 - OD600)*155/0.1

Total soluble sugars and starch determination

Total soluble sugars were quantified using the anthrone method described by Irigoyen et al.[41]. Briefly, 100 μL of ethanolic extract was reacted with 3 mL of freshly prepared anthrone reagent and heated in a boiling-water bath for 10 s. After cooling, absorbance was measured at 625 nm. D-glucose standards at concentrations of 0, 62.5, 125, 250, 500, 1000, and 2000 mg L^{-1} prepared in 80% ethanol were processed identically, and sugar concentrations were calculated using the resulting standard curve.

Shoot starch content was determined according to the method described by Marshall[42]. Residues remaining after proline and soluble sugar extraction were air-dried for 1–2 d and

extracted with 5 mL of 1.1% HCl at 100 °C for 30 min. After filtration, 1 mL of the extract was mixed with 5 mL of freshly prepared anthrone reagent, cooled on ice, heated at 100 °C for 11 min, and cooled again. Absorbance was then recorded at 630 nm, and starch concentration was calculated using the D-glucose standard curve described above. Anthrone reagent was prepared by dissolving 1 g of anthrone in 500 mL of 72% H_2SO_4.

Total soluble protein and antioxidant enzyme activities

Total soluble protein was extracted from 1 g of fresh leaf tissue by homogenization in liquid nitrogen with 100 mg of PVP, followed by extraction with 3 mL of 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM sodium metabisulfite. The homogenate was centrifuged at 15,000 rpm for 25 min at 4 °C, and the supernatant was collected, aliquoted, frozen in liquid nitrogen, and stored at −80 °C until analysis. All extraction procedures were performed at 4 °C.

Protein concentration was determined using the Bradford assay[43] with bovine serum albumin (BSA; 50–1000 μg mL^{-1}) as the standard. Briefly, 20 μL of extract or standard was mixed with 1 mL of Bradford reagent, incubated for 5 min at room temperature, and absorbance was measured at 595 nm using a spectrophotometer. Protein concentrations were calculated from the BSA standard curve.

Peroxidase (POD) activity was determined according to Hemeda and Klein[44]. Enzyme extracts were prepared in 0.5 M phosphate buffer (pH 7.4). The reaction mixture contained 1.5 mL of 0.1 M sodium phosphate buffer, 100 μL of 1% guaiacol, and 100 μL of 1% H_2O_2 in a 3-mL quartz cuvette. The reaction was initiated by adding 100 μL of enzyme extract, and the increase in absorbance at 470 nm was recorded over a 3-min period using a spectrophotometer. POD activity was calculated from the rate of change in absorbance over time.

Catalase (CAT) activity was determined according to Chance and Maehly[45]. Briefly, 1.5 mL of sodium phosphate buffer and 100 μL of H_2O_2 (1000 mM) were mixed in a 3-mL quartz cuvette. The reaction was initiated by adding 100 μL of enzyme extract, which was maintained on ice before use. After gentle mixing, the decrease in absorbance at 240 nm was monitored spectrophotometrically over a 3-min period. CAT activity was calculated from the rate of H_2O_2 decomposition based on the change in absorbance at 240 nm over time.

Superoxide dismutase (SOD) activity was determined using the photochemical inhibition of nitroblue tetrazolium (NBT) reduction, following Beauchamp and Fridovich[46]. Briefly, 0.5 g of fresh leaf tissue was homogenized on ice in phosphate buffer (pH 7.8), and the homogenate

was centrifuged to obtain a clear supernatant used as the crude enzyme extract. The reaction mixture contained 100 µL of phosphate buffer (0.1 M, pH 7.5), 100 µL of NBT, 200 µL methionine (0.25 M), 150 µL riboflavin (1 mM), and 1.0 mL of calcium carbonate (200 mM), together with 400 µL of enzyme extract. For the control, enzyme extract was replaced with an equivalent volume of buffer. One control tube was kept in the dark and used as the blank. The remaining reaction mixtures were illuminated under a fluorescent lamp for 10–20 min, after which absorbance was measured at 560 nm. One unit of SOD activity was defined as the amount of enzyme required to inhibit NBT reduction by 50% relative to the illuminated control. SOD activity was expressed as U g^{-1} fresh weight (FW).

Leaf mineral nutrient analysis

Plant tissues were washed thoroughly with tap water, followed by washing with 0.1 M HCl to remove surface contaminants, and finally rinsed with distilled water. Samples were oven-dried at 70 °C for 48 h, ground to a fine powder, and used for nutrient analysis.

Total nitrogen (N) was determined using the Kjeldahl method[47]. Approximately 1.0 g of dried, ground tissue was digested with 20 mL of concentrated H_2SO_4 and one Kjeldahl digestion tablet. Following digestion, the digest was subjected to steam distillation after the addition of NaOH, and the released ammonia was trapped in 2–4% boric acid containing bromocresol green and methyl red indicators. The trapped ammonia was titrated with 0.1 N HCl, and total N concentration was calculated.

Phosphorus (P), potassium (K), and calcium (Ca) concentrations were determined by inductively coupled plasma–optical emission spectrometry (ICP-OES; Optima 8300, PerkinElmer) following sample preparation by dry ashing and acid dissolution (PerkinElmer, 1982). Briefly, accurately weighed dried plant material was gradually heated from 100 to 300 °C on a hot plate for carbonization and subsequently ashed at 450 °C for 24 h. The resulting ash was dissolved in 5 mL of 6 M HCl. After evaporation of the hydrochloric acid, the residue was dissolved in 2% HNO_3, filtered, and brought to a final volume of 50 mL with 2% HNO_3. Elemental concentrations were then determined by ICP-OES.

Statistical analysis

The experiment was conducted as a two-factor factorial experiment in a completely randomized design with four replicates. Data were analyzed using SAS 9.4, and treatment means were separated using Duncan's multiple range test at $P \leq 0.05$. Pearson's correlation

290 analysis, principal component analysis (PCA), and hierarchical cluster analysis were also
291 performed to evaluate relationships among measured traits and multivariate patterns of
292 treatment responses.

293 **Results**

294 **Turf quality and growth parameters**

295 As shown in Figure 1, plants grown under the optimum temperature exhibited the highest
296 visual quality. However, no significant differences were observed among the 0.5, 1, and 2 mM
297 GABA treatments, whereas the lowest visual quality was recorded in plants that did not receive
298 GABA foliar application. Plants exposed to heat stress exhibited lower visual quality. Under
299 heat-stress conditions, increasing the GABA concentration up to 1 mM significantly improved
300 visual quality; however, a further increase in GABA concentration to 2 mM significantly
301 reduced plant visual quality (Figs 1 and 2a).

302 The results showed that fine-leaved fescue plants grown under the optimum temperature
303 had longer shoots than those exposed to heat stress. Under the optimum temperature, increasing
304 the GABA concentration increased shoot length from 23 to 29 cm. Heat stress significantly
305 reduced shoot length in both GABA-treated and untreated plants. Under heat-stress conditions,
306 shoot length increased progressively with increasing GABA concentration up to 1 mM;
307 however, a further increase in GABA concentration to 2 mM significantly reduced shoot length
308 (Fig. 2b).

309 Heat stress significantly reduced shoot fresh and dry weights compared with those under the
310 optimum temperature. GABA application alleviated these reductions, with the greatest
311 improvement generally observed at 1 mM under heat stress; although 2 mM GABA was less
312 effective, both concentrations maintained higher shoot biomass than the untreated control.
313 Under optimal conditions, shoot fresh and dry weights increased with increasing GABA
314 concentration (Figs. 2c and d).

315 Heat stress significantly reduced root fresh and dry weights, whereas GABA application
316 improved both parameters, with the most consistent responses observed at 1 mM under heat
317 stress. Under optimum temperature conditions, root fresh and dry weights increased with
318 GABA application, although no significant differences were observed between the 1 and 2 mM
319 treatments (Figs. 2e and f).

320 As shown in Figure 2g, under the optimum temperature, plants foliar-sprayed with 2 mM
321 GABA exhibited significantly greater root length than those treated with the other GABA

concentrations. However, no significant difference was observed between the 0.5 and 1 mM GABA treatments. In contrast, heat stress significantly reduced root length in plants that did not receive GABA foliar application. Nevertheless, increasing GABA concentration progressively increased root length compared with the untreated control.

Heat stress significantly reduced the root-to-shoot fresh weight ratio at the 1% significance level compared with the optimum temperature. However, under heat-stress conditions, foliar application of GABA significantly increased the root-to-shoot fresh weight ratio up to 1 mM, followed by a non-significant decrease. The ratio at 2 mM did not differ significantly from those at 0.5 and 1 mM. Under the optimum temperature, the root-to-shoot fresh weight ratio showed an increasing trend with increasing GABA concentration, with the highest ratio recorded in plants foliar-sprayed with 1 mM GABA. However, no significant difference was observed between the 2 and 0.5 mM GABA treatments (Fig. 2h).

Photosynthetic pigment content

The results of the interaction between temperature regimes and GABA concentrations showed that heat stress significantly reduced total leaf chlorophyll content in fine-leaved fescue by approximately 53% compared with that under optimum temperature. In contrast, GABA-treated plants exhibited higher total chlorophyll content under both optimum and heat-stress conditions. Increasing the GABA concentration progressively enhanced total leaf chlorophyll content under both temperature regimes, with the highest chlorophyll content recorded at 2 mM GABA. The lowest total chlorophyll content was observed in plants that received no GABA foliar application under heat-stress conditions (Fig. 3a).

The results of the interaction between temperature regimes and GABA concentrations showed that heat stress significantly reduced total carotenoid content in the leaves of fine-leaved fescue. In contrast, foliar application of GABA significantly increased total carotenoid content under the optimum temperature compared with heat-stress conditions. Increasing the GABA concentration enhanced total leaf carotenoid content under both optimum and heat-stress conditions, with the highest content recorded at 2 mM GABA. Notably, plants exposed to heat stress and foliar-sprayed with 1 or 2 mM GABA showed the highest retention of total leaf carotenoid contents among the heat-stressed plants, with no significant difference between these two treatments (Fig. 3b).

Leaf water status and membrane stability

The results of the mean comparison (Fig. 3c) showed that heat stress significantly reduced RWC by approximately 27.4% (1.3-fold decrease) compared with the optimum temperature. In contrast, all plants treated with different concentrations of GABA exhibited higher RWC under heat-stress conditions than the untreated control. Although RWC did not differ significantly among the different GABA concentrations, all GABA treatments differed significantly from the control. The highest RWC was recorded in plants foliar-sprayed with 0.5 and 1 mM GABA, whereas the lowest RWC was observed under heat stress without GABA foliar application.

Heat stress significantly increased EL by approximately 4.6-fold at the 1% significance level compared with the optimum temperature. However, under heat-stress conditions, foliar application of GABA significantly reduced EL at concentration up to 1 mM, with the lowest value being approximately 2.3-fold lower than that of the untreated control. No significant difference between the 1 and 2 mM GABA treatments. Under the optimum temperature, EL showed a decreasing trend with increasing GABA concentration, with the lowest EL observed in plants foliar-sprayed with 1 mM GABA; however, no significant difference was observed between the 1 and 2 mM GABA treatments (Fig.3d).

Starch and total soluble carbohydrate (TSC) content

Based on the results of the mean comparison (Fig. 3e), plants grown under heat-stress conditions had lower leaf starch content than those grown at the optimum temperature. However, foliar application of 0.5 mM GABA significantly increased leaf starch content under heat stress, whereas a further increase in GABA concentration to 1 and 2 mM resulted in a significant decrease in leaf starch content. Nevertheless, starch content in these treatments remained at levels not significantly lower than that of the untreated control. Under the optimum temperature, leaf starch content showed a slight decreasing trend with increasing GABA concentration compared with the control; however, no significant differences were observed between the control and the 0.5 and 1 mM GABA treatments (Fig. 3f).

Based on the results of the mean comparison (Fig. 3e), heat stress significantly increased total soluble carbohydrate (TSC) content in the leaves by approximately 117.8% (2.2-fold) compared with the optimum temperature. Under heat-stress conditions, foliar application of GABA increased leaf TSC content up to 1 mM; however, a further increase in GABA concentration to 2 mM resulted in a significant decrease in leaf TSS content in fine-leaved fescue. Under the optimum temperature, leaf TSS content also showed a significant increasing

388 trend with increasing GABA concentration up to 0.5 mM compared with the control, with no
389 significant difference between the 0.5 and 1 mM treatments. The highest leaf TSS content was
390 recorded in plants foliar-sprayed with 2 mM GABA, which differed significantly from the
391 control and all other GABA concentrations (Fig. 3e).
392

393 **Proline, malondialdehyde (MDA) content**

394 Heat stress significantly increased leaf proline content by 81.9% (approximately 1.8-fold)
395 compared with the optimum temperature. Under heat-stress conditions, foliar application of
396 GABA significantly increased leaf proline content at concentrations up to 1 mM; however, a
397 further increase in GABA concentration to 2 mM resulted in a significant decrease in proline
398 content in fine-leaved fescue leaves. Under the optimum temperature, leaf proline content also
399 showed a significant increasing trend with increasing GABA concentration up to 0.5 mM
400 compared with the control, although the 0.5 mM GABA treatment did not differ significantly
401 from the control or the 2 mM GABA treatment (Fig 3g).
402 Heat stress significantly increased MDA content by approximately sixfold compared with
403 the optimum temperature. Under heat-stress conditions, foliar application of GABA
404 significantly reduced MDA content as the GABA concentration increased; however, no
405 significant difference was observed between the 1 and 2 mM GABA treatments. Under the
406 optimum temperature, leaf MDA content also showed a decreasing trend with increasing
407 GABA concentration up to 1 mM compared with the control, followed by an increase at 2 mM
408 GABA. However, these differences among treatments were not statistically significant (Fig.
409 3h).
410

411 **Total soluble protein and antioxidant enzymes activity**

412 Heat stress reduced total soluble protein content in the leaves by approximately 13.3%
413 compared with the optimum temperature; however, this reduction was not statistically
414 significant. In contrast, total soluble protein content was maintained in plants foliar-sprayed
415 with 0.5 and 1 mM GABA under heat-stress conditions and increased significantly at 2 mM
416 GABA. Under the optimum temperature, total soluble protein content increased at 0.5 mM
417 GABA compared with the control, then declined at 1 mM to a level approximately similar to
418 that of the untreated plants, followed by a significant increase at 2 mM GABA (Fig. 4a).
419 Heat stress significantly increased leaf POD activity by 4.6-fold compared with the optimum
420 temperature. Under heat-stress conditions, POD activity reached its highest level in plants

foliar-sprayed with 0.5 mM GABA, followed by a decreasing trend at 1 and 2 mM GABA. Nevertheless, POD activity in plants exposed to heat stress remained higher than that in plants grown under the optimum temperature. Under the optimum temperature, the lowest leaf POD activity was recorded in the untreated control plants without GABA foliar application. Although POD activity also showed a decreasing trend with increasing GABA concentration under the optimum temperature, the differences among the GABA treatments and between these treatments and the control were not statistically significant (Fig. 4b).

Mean comparison results showed that heat stress significantly increased leaf CAT activity by approximately 3-fold compared with the optimum temperature. Under heat-stress conditions, foliar application of GABA increased CAT activity up to 1 mM; however, a further increase in GABA concentration to 2 mM resulted in a significant decrease in leaf CAT activity in fine-leaved fescue. CAT activity at 2 mM GABA did not differ significantly from that at 0.5 mM GABA or from that of the untreated control. Under the optimum temperature, CAT activity also showed an increasing trend with increasing GABA concentration compared with the control, although the 2 mM GABA treatment did not differ significantly from the other treatments. In addition, CAT activity at 0.5 mM GABA did not differ significantly from the control (Fig. 4c).

Heat stress significantly increased leaf SOD activity by approximately 1.6-fold (55.6%) compared with the optimum temperature. Under heat-stress conditions, foliar application of GABA significantly increased SOD activity by approximately 40% compared with the untreated control, with the maximum increase observed at 1 mM GABA. However, a further increase in GABA concentration to 2 mM resulted in a significant decrease in SOD activity. Under the optimum temperature, SOD activity also showed a significant increasing trend with increasing GABA concentration compared with the treatment without foliar application, with the 1 mM GABA treatment exhibiting higher SOD activity than the other treatments (Fig. 4d).

Leaf mineral concentrations

Heat stress significantly reduced leaf nitrogen content by approximately 39.2% compared with the optimum temperature. Under heat-stress conditions, foliar application of GABA increased leaf nitrogen content up to 1 mM, followed by a decrease at 2 mM; however, no significant differences were observed among the three GABA concentrations. Under the optimum temperature, leaf nitrogen content also showed an increasing trend with increasing

GABA concentration compared with the control, although no significant differences were observed among the three GABA concentrations (Table 1).

Heat stress significantly reduced leaf phosphorus content by approximately 42.2% compared with the optimum temperature. Under heat-stress conditions, foliar application of GABA increased leaf phosphorus uptake up to 1 mM, followed by a decrease at 2 mM; however, the difference between the 1 and 2 mM GABA treatments was not statistically significant. Under the optimum temperature, leaf phosphorus content also showed a slight increasing trend with increasing GABA concentration up to 1 mM compared with the control. At 2 mM GABA, however, the increase in leaf phosphorus content was significantly higher than that in the control (Table 1).

Heat stress significantly reduced leaf potassium content by approximately 39.7% compared with the optimum temperature. Under heat-stress conditions, foliar application of GABA increased leaf potassium content up to 1 mM, followed by a decrease at 2 mM; however, the difference between the 1 and 2 mM GABA treatments was not statistically significant. Under the optimum temperature, leaf potassium content also showed an increasing trend with increasing GABA concentration up to 1 mM compared with the control, followed by a slight decrease at 2 mM GABA. Nevertheless, no significant difference was observed between the 1 and 2 mM GABA treatments (Table 1).

Heat stress significantly reduced leaf calcium content by approximately 46.7% compared with the optimum temperature. Under heat-stress conditions, foliar application of GABA increased leaf calcium content up to 1 mM concentration, followed by a slight decrease at 2 mM; however, no significant difference was observed between the 1 and 2 mM GABA treatments. Under the optimum temperature, leaf calcium content also showed a very slight increasing trend with increasing GABA concentration up to 1 mM compared with the control, followed by a slight decrease at 2 mM GABA. Nevertheless, the differences among the treatments were not statistically significant (Table 1).

Multivariate analysis

Pearson correlation analysis revealed strong coordination among growth, water status, photosynthetic pigments, membrane stability, antioxidant defense, and mineral nutrition (Fig. 5). Growth-related traits were strongly and positively correlated with one another and with RWC, particularly SFW, SDW, RFW, and RFW/SFW ($r = 0.89–0.98$). RWC was also strongly associated with photosynthetic pigments and biomass, indicating that maintenance of tissue

hydration was closely linked to growth and photosynthetic capacity. TChl and carotenoids were highly correlated (r = 0.98) and showed positive associations with growth and RWC but negative relationships with EL and MDA. Similarly, N was strongly correlated with biomass, RWC, and photosynthetic pigments (r = 0.86–0.97), whereas P, K, and Ca were strongly interrelated (r = 0.87–0.96), suggesting coordinated maintenance of nutritional status under heat stress. In contrast, EL and MDA exhibited a very strong positive correlation (r = 0.98) and were negatively associated with most growth- and physiological-performance traits, indicating a close relationship between membrane destabilization and lipid peroxidation. The stress-response variables also formed a distinct cluster, with strong correlations among proline, TSC, SOD, and CAT (r = 0.93–0.97), suggesting coordinated osmotic adjustment and antioxidant defense.

Hierarchical clustering revealed differences in the physiological and biochemical profiles of the optimum-temperature (OT) and heat-stress (HS) (Fig. 6). OT treatments were characterized by higher standardized values for biomass, RWC, visual quality, photosynthetic pigments, and N, whereas untreated heat-stressed plants (HSG0) showed the opposite pattern, together with higher EL and MDA. Foliar GABA markedly modified the physiological response to heat stress. Among the HS treatments, 1 mM GABA (HSG1) showed the strongest shift toward the OT profile, particularly for biomass, RWC, chlorophyll, carotenoids, and N. The 0.5 and 2 mM treatments produced intermediate responses. In contrast, HSG0 remained distinctly separated and exhibited the strongest stress-associated profile. Stress-related traits, particularly EL and MDA, clustered separately from growth and photosynthetic traits, while proline, SOD, CAT, and TSC showed coordinated responses under heat stress. Overall, the clustering pattern suggests that foliar application of GABA, particularly at 1 mM, was associated with improved growth, water status, photosynthetic traits, and membrane stability under heat stress, along with changes in stress-related responses.

Principal component analysis (PCA) cumulatively explained 85.4% of the total variance (PC1 = 60.3%, PC2 = 25.1%), revealing distinct physiological responses between the OTG and HSG treatment groups (Fig. 7). The OTG treatments displayed a dose-dependent shift along the PC1 axis, moving from high PC2 variance at lower concentrations (OTG0) toward a more defined state at higher concentrations (OTG2). In contrast, the HSG group separated strongly along PC1, with HSG0 acting as a distinct outlier while higher concentrations (HSG1 and HSG2) clustered tightly together, suggesting that increased active components override the base formulation's initial stress response. Overall, the clear separation between groups indicates that these treatments induce fundamentally different metabolic or physiological states, likely

driven by a major trait such as osmotic adjustment or secondary metabolite accumulation, warranting further biochemical analysis (Fig. 7).

Discussion

Abiotic stresses significantly affect plant growth and performance. Based on the present results, heat stress significantly reduced visual quality and impaired growth and physiological characteristics in fine-leaved fescue. The findings demonstrated that heat stress exerted extensive and interconnected effects on growth, plant water status, membrane stability, photosynthetic pigments, and metabolism in fine-leaved fescue. Significant reductions in visual quality, shoot length, shoot fresh and dry weight, root length, and root fresh weight under heat-stress conditions indicate that elevated temperature restricted not only shoot growth but also root development. Concurrently, the reduction in leaf relative water content (RWC), together with increased EL and MDA accumulation, indicated disruption of plant water status and increased membrane damage under heat stress. Therefore, the reductions in growth and visual quality observed in the present study can be considered the ultimate consequences of multiple physiological disturbances occurring simultaneously in response to heat stress. These findings are consistent with previous reports on tall and hard fescue[14,48], Lolium[49], and creeping bentgrass[50, 51,52,53].

Numerous studies have reported that plant growth regulators and natural plant-derived products can improve plant tolerance to abiotic stresses. GABA is a non-proteinogenic amino acid involved in various metabolic processes and can, to some extent, protect plants against abiotic stresses. GABA appears to provide partial protection against a range of abiotic stresses in many plant species by maintaining leaf turgor, increasing osmolyte accumulation, and reducing oxidative damage through enhancement of antioxidant defense mechanisms[24,54,55].

The present results showed that foliar application of 0.5 and 1 mM GABA significantly enhanced growth characteristics, including shoot and root length as well as shoot and root fresh and dry weight, under heat-stress conditions. In contrast, 2 mM GABA significantly reduced many of these traits, with the exception of total chlorophyll and carotenoid contents. This phenomenon is known as hormesis in stress physiology and suggests that GABA, acting as a signaling molecule, may activate defense pathways at relatively low concentrations, whereas at higher concentrations it may exert a stress-like effect[56]. This finding is consistent with the study of Li et al.[28] on creeping bentgrass, in which 0.5 mM GABA was identified as an effective concentration for inducing heat tolerance. In addition, the TCR-20 cultivar maintained higher

chlorophyll content under heat stress, whereas the Shikha cultivar exhibited higher chlorophyll content following foliar GABA application. Furthermore, histochemical staining confirmed increased oxidative stress under severe temperature conditions, while GABA effectively reduced the accumulation of hydrogen peroxide and superoxide in both creeping bentgrass cultivars[28].

Heat stress also reduced the root-to-shoot fresh weight ratio, with a decline of approximately 30–40% in the untreated control without GABA foliar application. However, foliar application of GABA increased this ratio up to 1 mM, suggesting that root development in fine-leaved fescue may be prioritized as part of the plant's adaptive response to heat stress. This response may be advantageous under stress conditions because a more robust root system can improve water and mineral uptake. This finding is consistent with Cheng et al.[50], who reported that GABA enhanced water uptake and osmotic regulation.

One of the most important findings of the present study was the approximately 4.6-fold increase in EL under heat-stress conditions. Increased EL generally indicates a decline in cell membrane stability and integrity and may result from enhanced production of reactive oxygen species (ROS) and peroxidation of membrane fatty acids. This interpretation is further supported by the approximately six-fold increase in MDA under heat stress observed in the present study, as MDA is a major end product of membrane lipid peroxidation and a widely used indicator of oxidative membrane damage. Accordingly, the concurrent increases in EL and MDA provide evidence of enhanced oxidative damage and disruption of cellular membrane structure in fine-leaved fescue exposed to high temperature. Thus, the present results clearly demonstrate that heat stress increased EL, indicating membrane damage, whereas GABA foliar application markedly reduced EL.

The role of GABA in strengthening this defense response was clearly evident in the present results. Under heat-stress conditions, GABA application up to 1 mM increased CAT and SOD activities while simultaneously reducing MDA accumulation and EL. These responses suggest a functional association among these traits, whereby enhancement of the antioxidant defense system in GABA-treated plants may have restricted ROS accumulation, thereby reducing lipid peroxidation and subsequent membrane damage. The protective effects of GABA have been associated with enhanced activities of antioxidant enzymes, including SOD, POD, and APX, which contribute to ROS detoxification[57]. as well as modulation of heat-shock factor (HSF) signaling pathways that promote the accumulation of heat-shock proteins (HSPs). Collectively, these responses may contribute to greater cellular membrane stability by limiting lipid peroxidation[32,33,58,59].

Based on the present results, heat stress reduced total chlorophyll and carotenoid contents by approximately 53% and leaf RWC by approximately 27.4%. In contrast, foliar application of GABA at 0.5 and 1 mM reduced the loss of chlorophyll and carotenoids and increased leaf RWC under heat-stress conditions, thereby potentially delaying leaf senescence and improving visual quality. The maintenance of membrane stability following GABA application was also likely associated with improved plant water status. Heat stress reduced leaf RWC by approximately 27.4%, whereas GABA application at all concentrations tested increased RWC compared with plants exposed to heat stress without GABA treatment. Reduced RWC under high-temperature conditions can lead to growth inhibition through decreased cellular turgor, restricted cell expansion, and consequently reduced development of both shoot and root tissues. Conversely, maintenance of higher RWC in GABA-treated plants may have provided more favorable conditions for continued cell division and expansion. The simultaneous increase in RWC and reduction in EL further suggest that GABA may contribute to the maintenance of both plant water status and cellular membrane integrity. These findings are consistent with previous reports in creeping bentgrass[60].

Enhanced GABA accumulation or metabolism may contribute to improved adaptation to abiotic stresses, including heat stress, across diverse plant species[58,61,62]. GABA catabolism produces glutamate, an important biosynthetic precursor of chlorophyll[63].

The significant reductions in leaf N, P, K, and Ca concentrations under heat stress, together with decreases in RWC, root growth, chlorophyll and carotenoid contents, and concomitant increases in EL and MDA, indicate that heat stress in fine-leaf fescue disrupts not only plant water status and redox homeostasis but also mineral nutrient homeostasis. The decline in N availability may be associated with restricted protein and photosynthetic pigment synthesis, thereby contributing to reductions in chlorophyll content and plant growth, whereas the decrease in P may limit the availability and transfer of energy required for metabolic processes and growth. Similarly, reduced K availability, given its central role in osmotic adjustment and maintenance of cellular turgor, may contribute to the decline in RWC. The reduction in Ca, owing to its essential functions in membrane stabilization and cellular signaling, may likewise be associated with increased EL and membrane damage. Thus, impaired nutritional status may represent an additional factor exacerbating restrictions in root and shoot growth and reducing biomass accumulation under heat stress.

Exogenous application of GABA under heat stress promoted the recovery of mineral nutrient status, particularly at concentrations up to 1 mM. This response coincided with increased RWC, reduced MDA accumulation and EL, enhanced SOD and CAT activities, and

better preservation of photosynthetic pigments and plant growth. These coordinated responses suggest that improved mineral nutrition in GABA-treated plants may constitute an integral component of a broader cellular homeostatic response. In particular, improved K and Ca status may contribute to the maintenance of water balance and membrane stability, whereas enhanced N and P status may support the continuation of metabolic activity and growth. Consequently, the improvement in mineral nutrient status, together with strengthened antioxidant defense and osmotic regulation, may create more favorable conditions for maintaining the photosynthetic apparatus and dry matter accumulation, ultimately contributing to the preservation of the visual quality of fine-leaf fescue under heat stress.

Li et al.[52] investigated creeping bentgrass exposed to heat stress at 35/30 °C. Foliar application of 0.5 mM GABA significantly increased leaf N, P, and Ca concentrations in heat-stressed plants; in contrast, GABA did not increase K concentration under heat stress. Importantly, the increases in N, P, and Ca were accompanied by improvements in membrane stability, RWC, osmotic adjustment, chlorophyll content, photochemical efficiency, and photosynthesis. Thus, these findings are consistent with the results of the present study and further support the potential involvement of GABA in maintaining mineral nutrient homeostasis under heat stress.

Zarbakhsh and Shahsavar[64] investigated two pomegranate cultivars exposed to drought, salinity, and combined drought–salinity stress. Their results showed that stress reduced the concentrations of several mineral nutrients, whereas foliar application of GABA increased K concentration and the K/Na ratio, as well as Mg, P, Mn, Zn, and Fe concentrations. At the same time, GABA improved plant growth and photosynthetic capacity and reduced the uptake of the potentially toxic ions Na and Cl. These findings provide further evidence that, under osmotic and ionic stress conditions, GABA may contribute not only to osmotic adjustment and antioxidant defense but also to the maintenance of mineral nutrient homeostasis.

Conclusion

In summary, the results of the present study demonstrate that heat stress exerts multifaceted adverse effects on fine-leaved fescue, disrupting growth, water status, membrane integrity, photosynthetic pigment content, osmolyte metabolism, antioxidant defense, and mineral nutrient homeostasis. Foliar application of GABA, particularly at concentrations of 0.5 and 1 mM, ameliorated many of these adverse effects through a coordinated series of physiological and biochemical responses. The hormetic response observed, with beneficial effects at low to

moderate concentrations and adverse effects at higher concentrations, highlights the importance of optimizing GABA application rates for practical use in turfgrass management under heat stress conditions. The integrated improvements in antioxidant enzyme activities, membrane stability, water status, osmolyte accumulation, photosynthetic pigment retention, and mineral nutrient status in GABA-treated plants suggest that GABA acts through multiple interconnected pathways to enhance heat tolerance in fine-leaved fescue. These findings provide a mechanistic framework for understanding the protective role of GABA under heat stress and support the potential application of GABA as a cost-effective and environmentally friendly approach to improve turfgrass performance under high-temperature conditions.

Credit author statement and authors' contributions

Esmaeili S and Farrokhzad Y contributed equally to the conception and development of this work, including the methodology, investigation, data analysis, and interpretation of the results. Both authors participated in manuscript preparation, critically revised the manuscript, and approved the final version for publication.

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Data Availability

All data supporting the findings of this study are available within the article.

Declarations

Ethics approval and consent to participate

Not applicable.

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Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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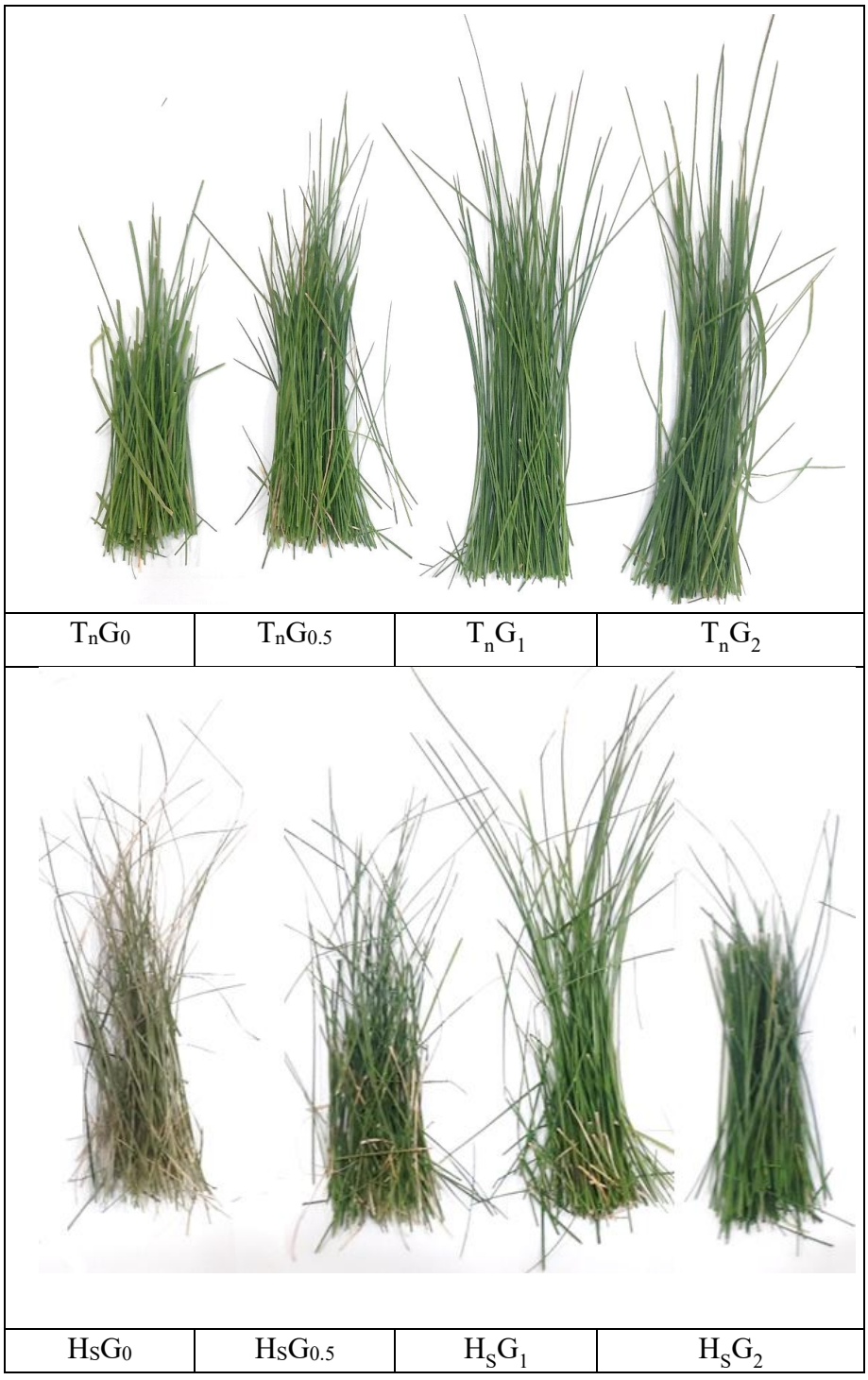

Fig. 1 An overview of the visual quality of fine-leaved fescue during 21 days of exposure to optimal temperature: Tn (23 °C day / 18 °C night) and heat stress: Hs (43 °C day / 37 °C night) at different GABA concentrations (G0: 0 mM; G0.5: 0.5 mM; G1: 1 mM; G2: 2 mM).

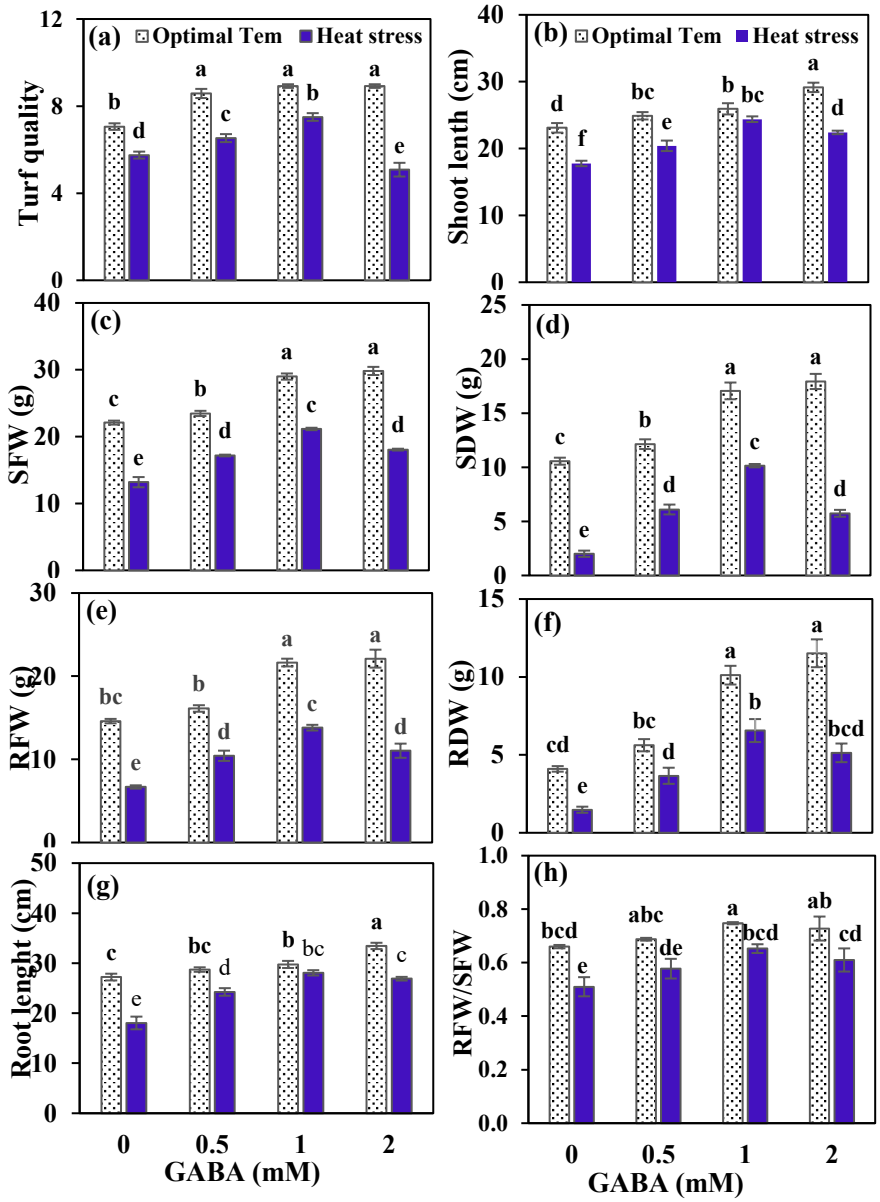

Fig. 2 Interaction effect of temperature and GABA on the (a) visual quality, (b) shoot length, (c) shoot fresh weight (SFW), (d) shoot dry weight (SDW), (e) root fresh weight (RFW), (f) root dry weight (RDW), (g) root length, root fresh weight to shoot fresh weight (RFW /SFW) of fine-leaved fescue over 21 days under optimal temperature (23 °C day / 18 °C night) and heat stress (43 °C day / 37 °C night). Means sharing common letters are not significantly different at the 5% probability level according to Duncan's multiple range test.

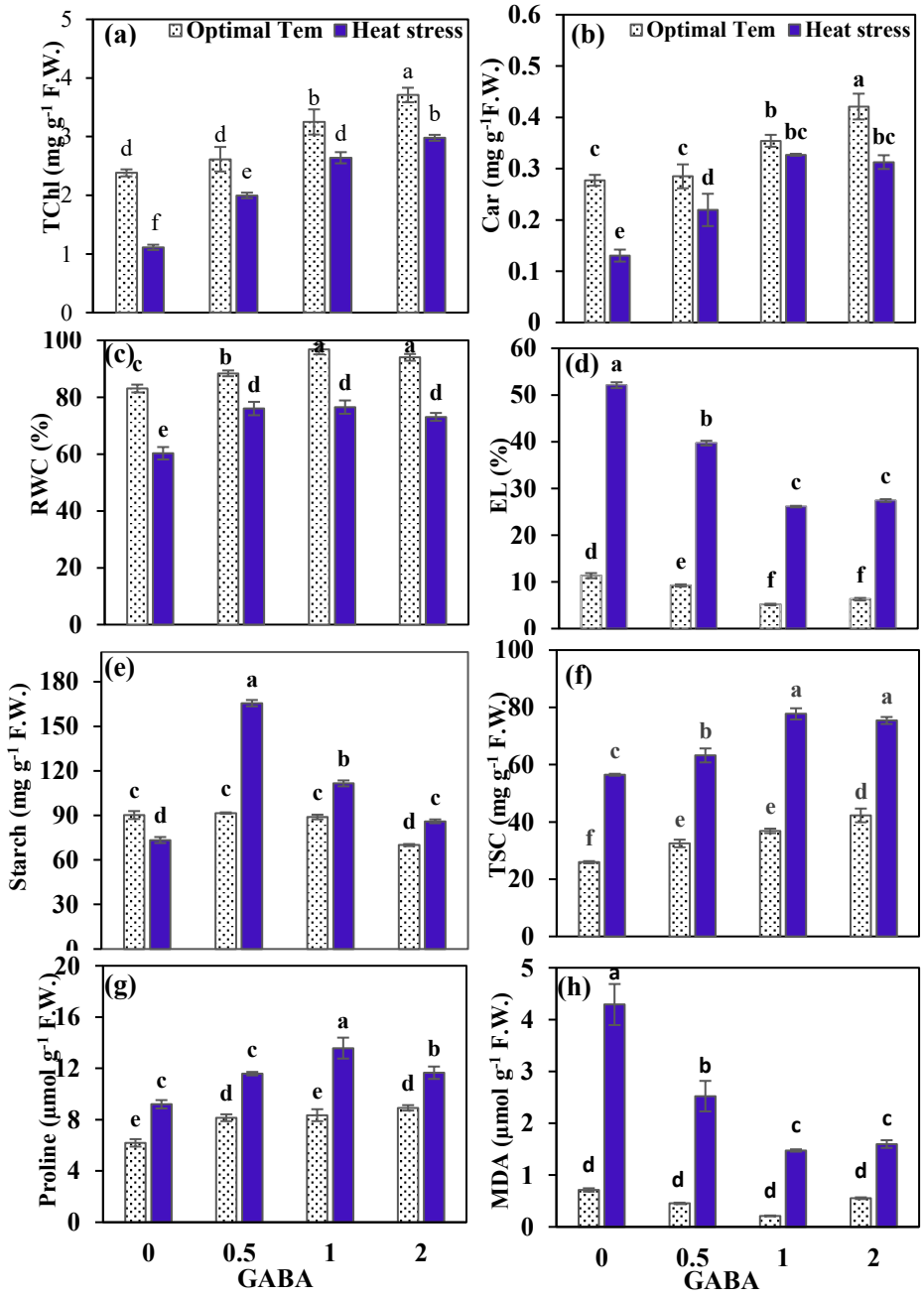

Fig. 3 Interaction effect of temperature and GABA on the (a) total chlorophyll (TChl), (b) carotenoids (Car), (c) relative water content (RWC), (d) electrolyte leakage (EL), (e) starch, (f) total soluble carbohydras (TSC), (g) proline, (h) malondialdehyde (MDA) of fine-leaved fescue over 21 days under optimal temperature (23 °C day / 18 °C night) and heat stress (43 °C day / 37 °C night). Means sharing common letters are not significantly different at the 5% probability level according to Duncan's multiple range test.

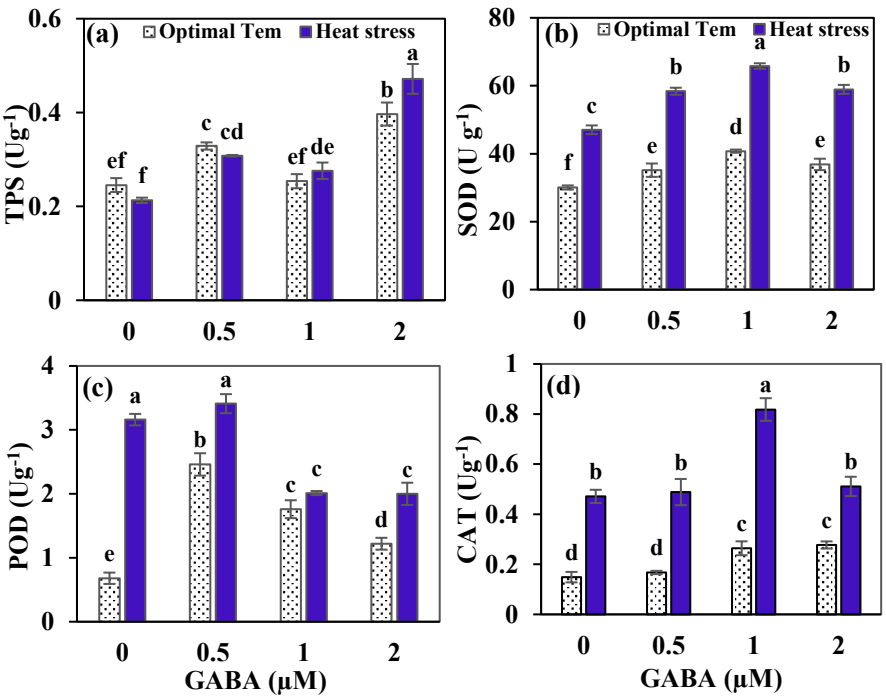

Fig. 4 Interaction effect of temperature and GABA on the (a) total soluble protein (TSP), (b) superoxide dismutase (SOD), (c) peroxidase (POD), and (d) catalase (CAT) of fine-leaved fescue over 21 days under optimal temperature (23 °C day / 18 °C night) and heat stress (43 °C day / 37 °C night). Means sharing common letters are not significantly different at the 5% probability level according to Duncan's multiple range test.

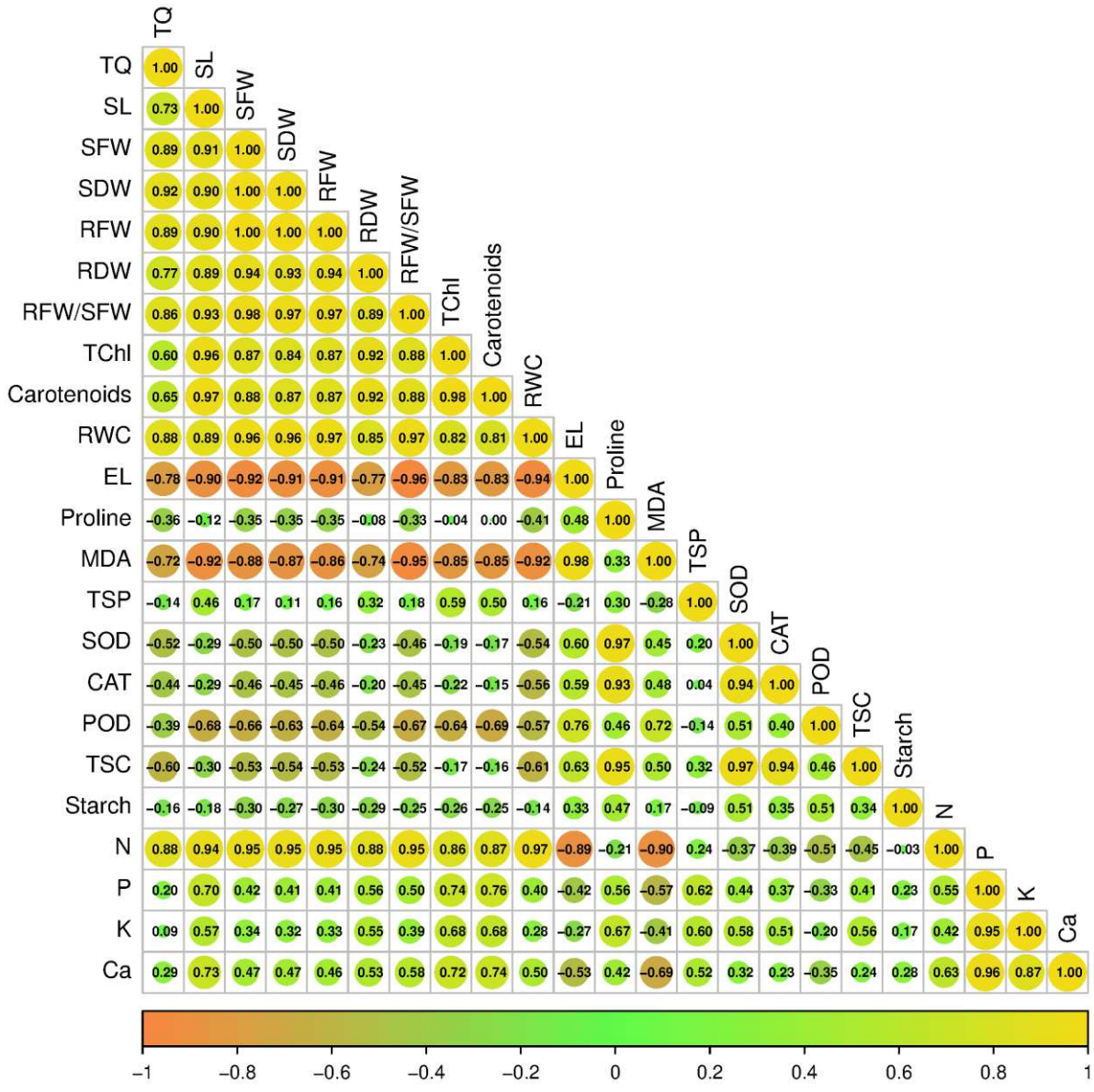

Fig. 5 Correlation heat map indicating correlations among visual quality, nutrients, morpho-physiological and biochemical traits of fine-leaved fescue under temperature and GABA treatments. Positive relationships are presented in light blue color and negative relationships are in red color. Abbreviations: total chlorophyll (TChl), relative water content (RWC), electrolyte leakage (EL), total soluble carbohydras (TSC), malondialdehyde (MDA), total soluble protein (TSP), (b) superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT).

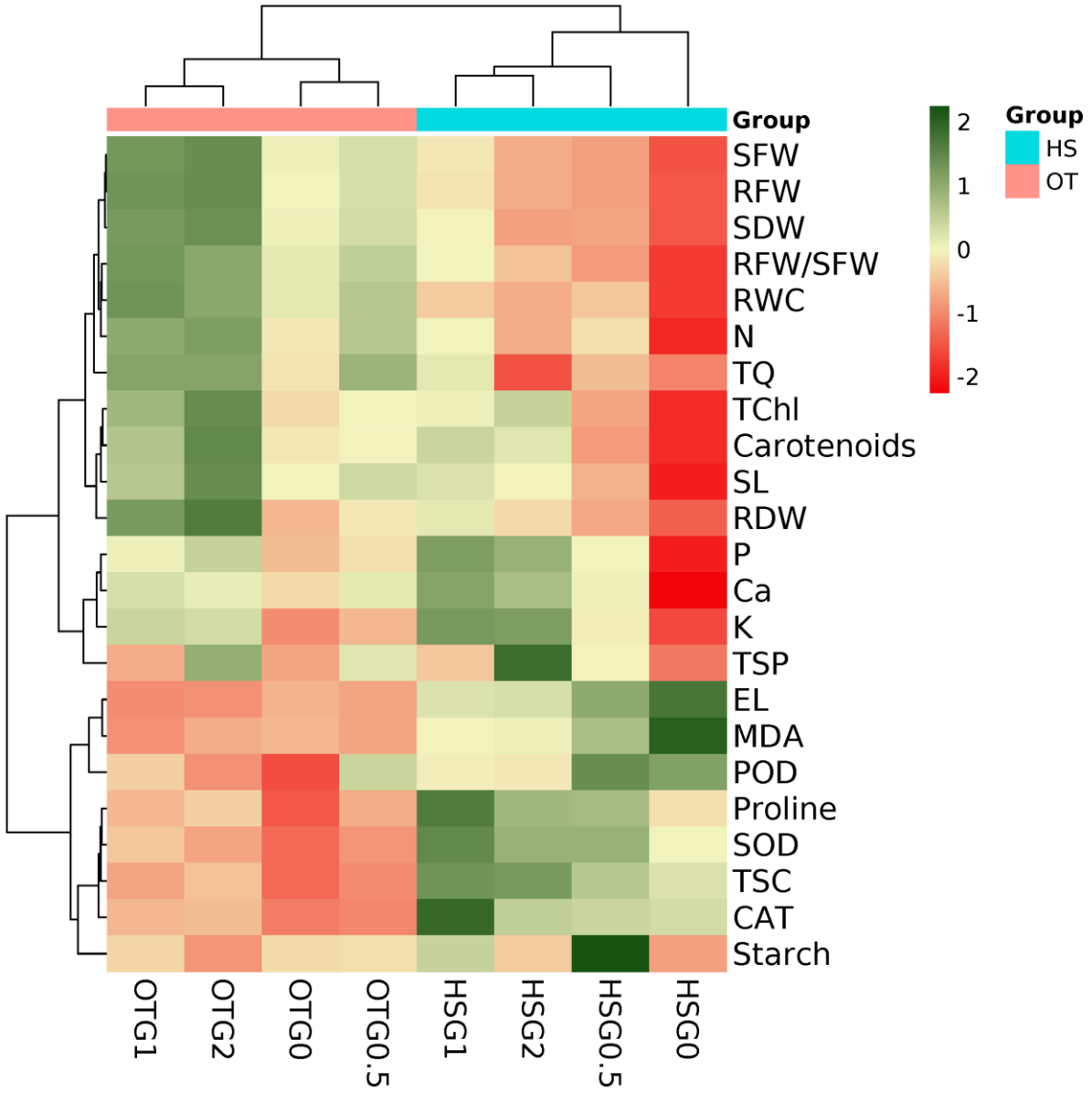

Fig. 6 Cluster analysis of treatments on fine-leaved fescue based on physical and chemical properties of the leaf. (gradient from low (red), medium (pink) to high (green). Abbreviations: total chlorophyll (TChl), relative water content (RWC), electrolyte leakage (EL), total soluble carbohydras (TSC), malondialdehyde (MDA), total soluble protein (TSP), (b) superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT).

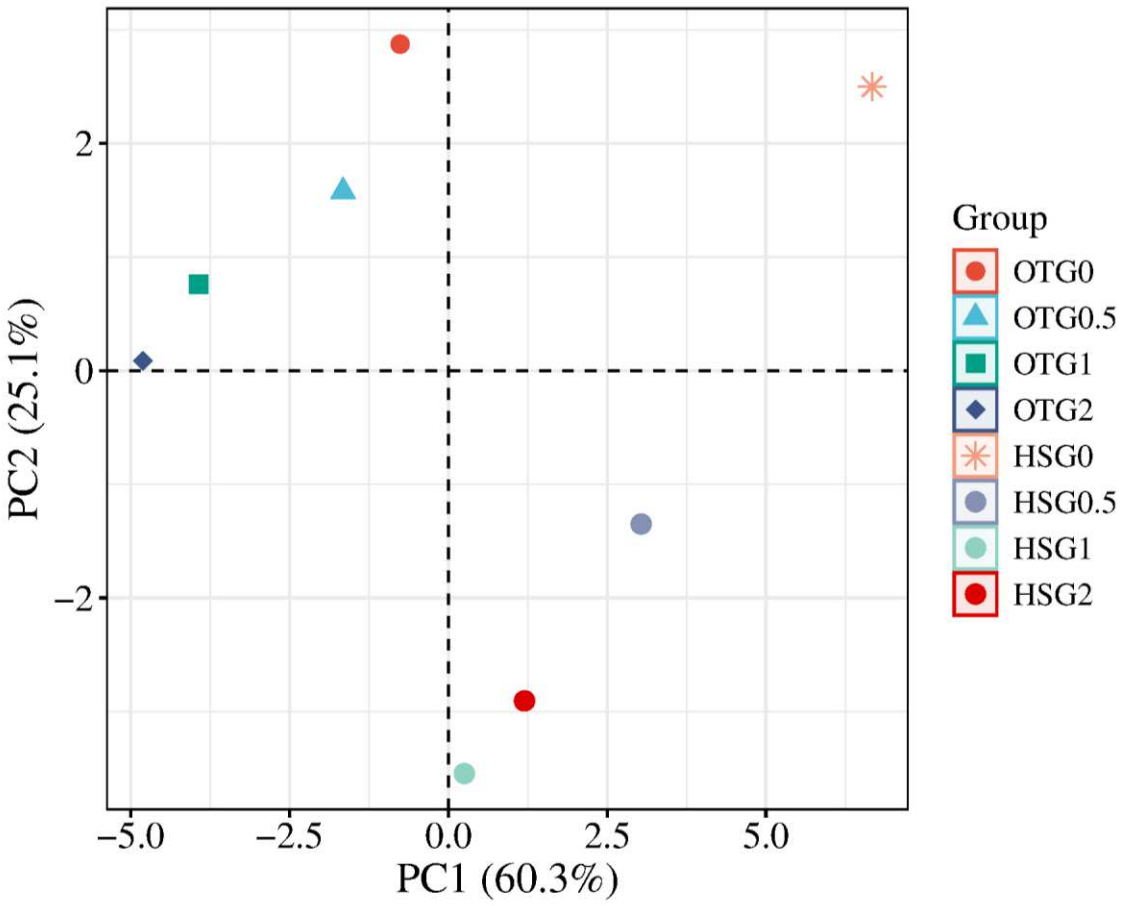

Fig. 7 Principal component analysis (PCA) of treatments on fine-leaved fescue. Abbreviations: OTG0: optimal temperature without GABA, OTG0.5: optimal temperature with 0.5 mg L^{-1} GABA, OTG1: optimal temperature with 1 mg L^{-1} GABA, OTG2: optimal temperature with 2 mg L^{-1} GABA, HSG0: heat stress without GABA, HSG0.5: heat stress with 0.5 mg L^{-1} GABA, HSG1: heat stress with 1 mg L^{-1} GABA, HSG2: heat stress with 2 mg L^{-1} GABA.

Table 1 Interaction effect of temperature and GABA on nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca) of fine-leaved fescue over 21 days under optimal temperature (23 °C day / 18 °C night) and heat stress (43 °C day / 37 °C night).

Temperature	GABA (µM)	N (%)	P (%)	K (%)	Ca (%)
Optimal	0	2.71±0.27^{cd}	0.34±0.022^{c}	2.44±0.10de	0.54±0.03
	0.5	3.12±0.013^{ab}	0.37±0.015^{bc}	3.03±0.27cd	0.61±0.01
	1	3.38±0.13^{a}	0.4±0.020^{bc}	4.59±0.23b	0.63±0.03
	2	3.49±0.17^{a}	0.43±0.050^{ab}	4.45±0.58b	0.60±0.03
Heat stress	0	1.65±0.269^{d}	0.2±0.004^{d}	1.47±0.20	0.22±0.04
	0.5	2.64±0.084^{c}	0.39±0.015^{bc}	3.84±0.15	0.59±0.06
	1	2.79±0.111^{bc}	0.5±0.024^{a}	5.77±0.44	0.76±0.03
	2	2.38±0.052^{c}	0.48±0.01^{a}	5.71±0.42	0.70±0.03

Means sharing common letters are not significantly different at the 5% probability level according to Duncan's multiple range test.