

ABIOTIC STRESS RESPONSES AND BREEDING STRATEGIES FOR CLIMATE-RESILIENT FODDER CROPS: FUTURE PROSPECTS OF PEARL MILLET × NAPIER HYBRIDS

M. Vishnu¹, T. Ezhilarasi^{2*}, R. Pushpam³, K. Chandrakumar⁴ and A. Senthil⁵

¹ Department of Genetics and Plant Breeding, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India

^{2,3} Department of Forage crops, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore-641003, Tamil Nadu, India

⁴ Department of Plant Molecular Biology and Bioinformatics, Centre for Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, Coimbatore -641003, Tamil Nadu, India

⁵ Agricultural Research Station, Tamil Nadu Agricultural University, Bhavanisagar-638451, Tamil Nadu, India

*Corresponding author: T. Ezhilarasi, *Email ID, tezhilarasitnau@gmail.com

ABSTRACT

Fodder crops provide vital nutrients for animal growth and milk production, these are critical to sustained livestock production. Because of its high biomass yield, excellent nutritional content, and broad adaptability, Pearl millet × Napier hybrid grass is a significant perennial fodder crop. However, the productivity and quality of fodder are severely limited by growing abiotic stresses like heat, salinity, and drought, due to climatic changes. These stresses have a negative impact on plant growth as well as physiological and biochemical processes. As a result, there is a decrease in crude protein content, change in the composition of fibre, decrease in digestibility, and accumulation of anti-nutritional factors, all of which have an impact on animal performance. Reduced biomass and protein content result from drought stress's disruption of water relations, photosynthesis, and nitrogen assimilation. Heat stress promotes leaf senescence, oxidative damage, and metabolic disruptions, lowering the quality of forage, whereas salinity stress results in osmotic imbalance and ion toxicity, which compromise nutrient uptake and membrane stability. Osmotic adjustment, the buildup of compatible solutes, antioxidant defense mechanisms, and morphological changes are how forage crops react. The review also covers both traditional and cutting-edge breeding techniques, such as germplasm screening, direct and indirect selection, introgression of stress-tolerant genes, molecular breeding, and biotechnological methods, for enhancing abiotic stress tolerance in Pearl millet x Napier hybrids. These tactics can promote year-round fodder production and increase climate resilience.

KEYWORDS: Abiotic stress; Forage growth; Drought; Salinity; Heat stress; Breeding strategies

1. INTRODUCTION

The backbone of Asian agriculture is crop-livestock systems. Beyond food production, livestock rearing is an important component of Asian agriculture (1). Livestock is the second largest sector and an essential part of nation's agriculture (2). Animal products such as milk and meat can be produced year-round and are important sources of protein and essential amino acids. Livestock production also increases income, improves economic stability, creates employment, supports rural livelihoods, and contributes to poverty reduction (1). Among the different livestock production systems, mixed crop-livestock systems are particularly important because they sustain a large proportion of the world's poor population and produce a major share of global ruminant livestock (3). Grazing fields account for up to 40% of India's total land area (4). However, for the past 20 years, the total area under fodder cultivation has remained relatively constant at 8.4 mha (5.23%) (4, 5). Approximately 54% of the cultivated fodder area is under sorghum and berseem, grown on 2.6 and 1.9 mha, respectively (4, 6). Fodder crops are often grown under stressful and unproductive conditions, resulting in lower-quality herbage (7). Although fodder crops have wide adaptability, can grow under stress, and provide regular income and employment, their storage, transport, processing, and conservation remain challenging (4). The pearl millet × Napier hybrid combines the deep roots and perennial nature of Napier with the palatability of pearl millet. It is suitable for year-round fodder production because of its high productivity and palatability. On a dry matter basis, it contains 7-10% crude protein, 28-30% crude fibre, and 10-11.5% ash. Once established, it can provide 8-10 cuts annually and produce fodder for 5-6 years under proper management (8). Adequate quantities of good-quality feed and fodder are essential for increasing cattle productivity (4). Despite improvements in feed and fodder availability over the last decade, a gap remains between demand and availability, particularly during lean periods, droughts, and abnormal weather conditions (4). India's fodder cultivation area has remained largely unchanged for two decades and is insufficient to meet the requirements of its growing livestock population (9). Poor soil fertility, low soil moisture, and prolonged dry periods during crop growth are major constraints to fodder productivity in arid and semi-arid regions (7).

Abiotic stresses severely affect crop growth, development, productivity, and quality. These stresses arise from unavoidable environmental conditions and can substantially reduce crop productivity (10). Climate change, including rising temperatures and atmospheric CO₂ levels, can reduce both dry matter production and the nutritional quality of forage crops (11). Individual or combined abiotic stresses disrupt signalling pathways, gene functioning, and plant growth and development, potentially reducing crop productivity by 50-70% (11, 12). Drought and heat stress particularly affect vegetative growth and fodder yield, while salinity is an increasing constraint to agricultural productivity, especially in coastal regions (13-15). Drought and salinity significantly affect plant height, tillering, leaf number and area, internode number, and fresh and dry biomass of shoots and roots. They also reduce chlorophyll, carotenoids, photosynthesis, transpiration, stomatal conductance, membrane stability, and protein content, while increasing reactive oxygen species that damage chlorophyll, proteins, DNA, and cell membranes. Salinity further causes osmotic stress and ion toxicity, ultimately reducing the productivity and nutritional quality of green fodder crops (11). These stresses can reduce farmers' income and affect livestock productivity and food security through reduced fodder availability and milk production. Therefore, improving abiotic stress tolerance in pearl millet × Napier hybrid grass is necessary to address drought, heat, and salinity stresses. Developing climate-smart fodder crops can support year-round fodder production and contribute to increased milk and livestock production. This review aims to facilitate the development of abiotic stress-tolerant fodder crops by discussing the effects of abiotic stresses, plant responses to these stresses, and approaches for improving stress tolerance in fodder crops.

2. Impact of abiotic stresses

2.1. Impact of drought stress on plant growth

2.1.1. Germination and seedling growth

In fodder sorghum, drought increased the root-to-shoot ratio but reduced germination percentage, seedling length, biomass, and vigour index relative to the control (16), showing how limited water availability impedes early germination and seedling growth.

2.1.2. Morphological traits

Water stress reduces plant height, tiller number, leaf area, fodder yield, shoot production, fresh weight, and dry matter across fodder species, including Pearl millet Napier hybrid (17), Rhodes grass (18), fodder sorghum (19), (20), (21), *Festuca arundinacea* (22), alfalfa (23), (24) fodder maize (25), Napier grass (26), berseem clover (27) and berseem (28), buffel grass (29), and cool-season grasses (30),(31). Root responses are more variable: while shoot and total biomass generally fall, the root-to-shoot ratio often rises as plants prioritise root growth to access water, as seen in alfalfa (23), (32) and *Cenchrus ciliaris* and *Cyperus arenarius* (33), though severe drought ultimately reduces root dry weight too, as in tall fescue (34), *Festulolium braunii* (35) and perennial ryegrass (36), (37). By restricting cell division, photosynthesis, and leaf expansion, drought lowers plant height, shoot growth, and forage quality; moderate stress can improve the root-to-shoot ratio for water uptake, but severe or prolonged drought damages roots irreversibly. These impacts are summarized in Table 1.

2.1.3. Nutritional quality

Drought reduces fibre digestibility in some species while altering fibre composition differently across others: ADF and NDF fell in water-stressed forage grasses (30), yet increased alongside declining in vitro digestibility in fodder sorghum (21). Crude protein content typically declines under drought, as in lucerne (38), fodder maize (25), Rhodes grass (18), and *Pennisetum glaucum* (39), reflecting reduced protein biosynthesis and increased degradation, though moderate irrigation deficits can raise crude protein, ash, digestibility, and fibre in forage sorghum while lowering ADF, NDF, and protein yield (20). Reduced leaf area and photosynthesis under drought lower cellulose, hemicellulose, and fibre production; mild stress can raise soluble carbohydrate and protein levels, but severe drought depletes protein reserves, and lignification or a lower leaf-to-stem ratio can raise ADF and NDF even as cell-wall synthesis declines.

2.1.4. Physiological and biochemical changes

Drought consistently lowers chlorophyll and photosynthetic pigment content, as in *Agropyron desertorum* (40), *Poa pratensis* (41), and *Cenchrus ciliaris* and *Cyperus arenarius* (33), through oxidative damage from drought-induced reactive oxygen species that preferentially degrade chlorophyll a in the photosystem reaction centre; alfalfa showed the same pattern for chlorophyll a even as chlorophyll b and total chlorophyll increased (23). Photosynthetic rate, transpiration, and stomatal conductance decline in parallel across species including *Festulolium*

Table 1. Morphological responses of fodder crops to drought stress

Crop species	Increased	Decreased	References
Fodder sorghum (<i>Sorghum bicolor</i>)	Root to shoot ratio	Germination percentage, seedling length, seedling biomass, seedling vigour index	(16)
Pearl millet Napier hybrid	-	Plant height, number of tillers, leaf area, green fodder yield, dry fodder yield	(17)
<i>Festuca arundinacea</i>	-	Shoot production	(22)

Alfalfa (<i>Medicago sativa</i>)	Root biomass, root to shoot ratio	Plant height, shoot biomass	(23)
Fodder maize (<i>Zea mays</i>)	-	Stem fresh weight, leaf fresh weight, dry matter yield	(25)
Fodder sorghum	-	Plant height, fresh weight, dry weight	(21)
Napier grass (<i>Pennisetum purpureum</i>)	-	Plant height, leaf width, dry matter weight	(26)
Berseem clover	-	Plant height, total biomass, dry weight	(27)
Berseem	-	Fresh fodder yield	(28)
Buffel grass	-	Shoot dry weight	(29)
<i>Cenchrus ciliaris</i> and <i>Cyperus arenarius</i>	-	Shoot fresh weight, shoot dry weight, shoot length, root biomass	(33)
Tall fescue (<i>Festuca arundinacea</i>)	-	Root dry weight	(34)
Alfalfa	Root to shoot ratio	Total biomass, shoot dry weight	(32)
Rhodes grass	-	Plant height, number of tillers, number of leaves, leaf area, green fodder yield, dry fodder yield	(18)
Fodder sorghum	-	Plant height, leaf length, leaf width, number of tillers, number of leaves, dry matter yield	(19)
Perennial ryegrass (<i>Lolium perenne</i>), Cocksfoot (<i>Dactylis glomerata</i>), Tall fescue, chicory (<i>Cichorium intybus</i>)	-	Leaf elongation rate, shoot dry weight, root dry weight	(37)
Tall fescue, Perennial rye grass	-	Root dry weight, root viability	(36)
<i>Elymus elongatum</i>	-	Shoot dry weight, leaf width	(42)
Fodder sorghum	-	Dry matter, stem weight, leaf to stem ratio	(20)
<i>Festulolium braunii</i> , tall fescue, perennial ryegrass	Root diameter, specific root length	Root length, root dry mass, shoot dry mass, total dry mass, root mass ratio	(35)
Tall fescue, Italian ryegrass, perennial ryegrass, meadow fescue	-	Dry matter yield	(30)
<i>Festuca pratensis</i> , <i>F. rubra</i> , <i>Lolium multiflorum</i> , <i>Lolium x hybridum</i>	-	Dry forage yield	(31)

braunii (22), tall fescue (34), fodder maize (24), Napier grass (26), *Poa pratensis* and *Lolium perenne* (43), berseem (27), berseem clover (28), lucerne (38), (44), *Urochloa* grass (45), and several cool-season grasses (30), (35), (36), (37), (42), (46), (47), generally alongside falling relative water content, membrane stability, and chlorophyll fluorescence (Fv/Fm). Fodder sorghum showed lower relative water content and membrane stability but more stomata under drought (16), (19). Stomatal closure limits transpiration and CO₂ uptake, further depressing photosynthesis while raising canopy temperature, sometimes first elevating evapotranspiration before it declines (30), (36). Drought-tolerant genotypes retain protective mechanisms such as improved cell-wall elasticity and membrane integrity (43), but these are overwhelmed under extreme, prolonged drought.

Drought also shifts carbon and nitrogen partitioning: shoot C:N ratio tends to fall as photosynthesis and CO₂ fixation decline, while roots accumulate more carbon and nitrogen and a higher C:N ratio as plants prioritise water uptake, as shown in *Festuca arundinacea* and *Festulolium braunii* (22), *Festuca arundinacea*, and *Lolium perenne* (35). Reduced soil moisture also promotes Na⁺ accumulation for osmotic adjustment at the expense of K⁺ and Ca²⁺ uptake, as seen in *Cenchrus ciliaris* and *Cyperus arenarius* (33); this supports short-term turgor maintenance but the resulting K⁺ deficit constrains growth, stomatal function, and enzyme activity.

2.2. Impact of salinity stress on plant growth

Salinity stress significantly impacts the growth, development, yield, and quality of fodder crops, disrupting plant metabolism and causing osmotic stress and ion toxicity.

2.2.1. Germination and seedling growth

Salinity restricts germination and early seedling growth across fodder species by reducing radicle and shoot length, as in fodder grasses generally (48) and in Italian ryegrass (49), *Bothriochloa pertusa*, *Dichanthium annulatum*, and *Panicum antidotale* (50). Germination percentage, seedling growth, and biomass fell similarly in *Euphorbia lathyris* (51), Rhodes grass (52), (53), and alfalfa (54) under rising salt concentrations. High salinity lowers soil osmotic potential and water uptake, while excess Na⁺ and Cl⁻ disrupt enzyme activity and cell division, impeding radicle and plumule formation; the resulting oxidative stress further reduces seedling fresh and dry weight. These impacts are summarized in Table 2.

2.2.2. Morphological traits

Salinity stress lowers plant height, tiller and leaf number, and fresh and dry biomass across most fodder species tested, including Napier grass (55), alfalfa (56), (57), (58) fodder sorghum (59), *Avena* species (60), *Euphorbia lathyris* (51), Rhodes grass (53), (52), Italian ryegrass (49) Pearl millet x Napier hybrid (61), and Marvel grass (50), (62). Root and shoot growth rates also decline, as in Italian ryegrass (49), though hybrid Giant Napier genotypes showed increased plant height, tillering, and fresh and fibrous root weight under moderate salt stress despite slower shoot initiation (63), and Napier grass dry matter yield increased with salinity even as fresh weight fell (64), (65). High salt concentrations reduce root water absorption, suppressing photosynthesis and growth and lowering green and dry fodder yield; dry matter content may rise or hold steady as tissues dehydrate, and salinity-tolerant genotypes sustain it by enhancing root growth. These impacts are summarized in Table 2.

2.2.3. Nutritional quality

Crude protein content generally falls under salinity stress, as in Napier grass (55), Italian ryegrass (49), Rhodes grass (52), (53), and Marvel grass (62), while ash, water-soluble carbohydrate, and crude fibre content often rise. Fibre fractions responded variably: NDF increased in Rhodes grass (53), while ADF, NDF, and ADL declined in Marvel grass (62), and cellulose and hemicellulose declined in Napier grass hybrids (65) while lignin stayed constant. Napier grass also showed reduced soluble but increased insoluble oxalate with rising salinity (64). By limiting nitrogen uptake through osmotic stress and ion toxicity, salinity reduces protein synthesis and restricts cellulose and hemicellulose synthesis via reduced cell division and water availability, though ash and soluble carbohydrate content typically rise through ionic accumulation and osmotic adjustment.

2.2.4. Physiological and biochemical changes

Salinity stress reduces chlorophyll a, b, total chlorophyll, and carotenoid content, along with their stability indices, in Pearl millet x Napier hybrids (61), Marvel grass (62), and Napier grass hybrids (65), though salt-tolerant Napier genotypes retain chlorophyll and carotenoid levels. This decline stems from ROS-driven oxidative damage to photosystems and pigment synthesis, offset in tolerant genotypes by stronger membrane stability and antioxidant defenses. Leaf relative water content, membrane stability, photosynthetic rate, transpiration, and stomatal conductance likewise fall under salinity in Pearl millet x Napier hybrids (61), Italian ryegrass (49), Marvel grass (62), Rhodes grass (52), and Napier grass hybrids (65), while stomatal resistance rises. SPAD values declined in Rhodes grass (53) and alfalfa (56) but increased in *Avena* species (60) alongside declining membrane stability and relative water content. Salinity lowers soil water potential and induces stomatal closure, restricting water and CO₂ uptake and thus photosynthesis and transpiration, while osmotic stress and ion toxicity damage membranes and raise electrolyte leakage; osmolyte accumulation and leaf thickening, however, can raise SPAD values.

Table 2. Morphological responses of fodder crops to salinity stress

Crop species	Increased	Decreased	References
<i>Bromus inermis</i> , <i>Elytrigia elongata</i> , <i>Puccinellia tenuiflora</i> , <i>Hordeum brevisubulatum</i> , <i>Kalidium gracile</i> , <i>Suaeda salsa</i>	-	Germination rate, radicle length, shoot length	(48)
Italian ryegrass	-	Germination rate, shoot growth rate, root growth rate	(49)
<i>Bothriochloa pertusa</i> , <i>Dichanthium annulatum</i> , <i>Panicum antidotale</i>	-	Germination percentage, radicle length, plumule length, biomass fresh weight, biomass dry weight	(50)
<i>Euphorbia lathyris</i>	-	Radicle fresh weight, hypocotyl fresh weight, germination rate, stem biomass, root biomass, total plant biomass	(51)
Rhodes grass	-	Germination percentage, root and seedling growth, increased root length, secondary root growth, plant height, leaf area, stem thickness, root length	(52)
Rhodes grass	-	Germination percentage, plant height, number of tillers, fresh weight, dry matter weight, shoot dry matter, root dry matter	(53)
Alfalfa	-	Germination rate	(54)
Napier grass	Dry matter content	Biomass yield, number of tillers, number of leaves, shoot initiation, plant height	(55)
Alfalfa	-	Relative growth rate	(56)
Fodder sorghum	-	Plant height, root fresh biomass, root dry biomass, yield	(59)

<i>Avena</i> sp.	-	Plant height, internode length, flag leaf length, flag leaf width, number of tillers, number of internodes, fresh weight, dry weight	(60)
Alfalfa	-	Fodder yield	(57)
Alfalfa	-	Shoot dry weight, root dry weight	(58)
Pearl millet x Napier hybrid	-	Root dry weight, shoot dry weight, root to shoot ratio	(61)
Marvel grass	-	Plant height, biomass	(62)
Napier grass	Dry matter content	-	(64)
Napier grass hybrid	Dry weight, dry matter content	Total fresh weight	(65)
Hybrid giant napier	Root development, root fresh weight	-	(63)

2.3. Impact of heat stress on plant growth

2.3.1. Morphological traits

Heat stress lowers germination rate, shoot fresh and dry weight, and biomass across fodder species, including Kentucky bluegrass, bent grass and perennial ryegrasses (66), fodder maize (67), forage oats (68), buffel grass (29), alfalfa (69), and perennial ryegrass, cocksfoot, tall fescue and chicory (37). Root activity, plant height, and biomass similarly decline in perennial ryegrass (70), *Chloris gayana* and *Setaria anceps* (71), *Setaria viridis* (72), *Agrostis* species (73), and tall fescue (36), (74), (75). By inhibiting enzymes and damaging embryonic tissue, heat stress reduces seed germination, cell division and elongation, root development, and overall biomass, while promoting leaf withering and chlorosis and, through elevated respiration, further depleting biomass. These effects are summarized in Table 3.

2.3.2. Nutritional quality

Heat stress raised starch, protein, crude fibre, and metabolizable energy in fodder maize through a concentration effect as biomass accumulation slowed (67), while lowering turf and lawn quality in perennial rye grass (70), Kentucky blue grass (47), and tall fescue (74). High temperatures generally promote chlorosis, senescence, and reduced tillering across fodder crops.

2.3.3. Physiological and biochemical changes

Heat stress lowers chlorophyll content, chlorophyll SPAD readings, relative water content, and photosynthetic efficiency across species including forage oats (68), alfalfa (69), perennial ryegrass and cocksfoot, tall fescue and chicory (37), fodder maize (67), and *Poa labillardierei* and related grasses (46), while increasing chlorophyll fluorescence and canopy temperature depression. Perennial rye grass showed reduced soil and leaf water content, photosynthetic rate, Fv/Fm, and membrane stability (36), and Kentucky blue grass showed reduced relative water content with increased electrolyte leakage and wilting (47), while tall fescue lost survival rate, chlorophyll content, and Fv/Fm (75). By disrupting water balance and increasing transpiration and

Table 3. Morphological responses of fodder crops to heat stress

Crop species	Increased	Decreased	References
Kentucky blue grass, bent grass, perennial rye grass	-	Germination rate	(66)
Fodder maize	-	Shoot fresh weight, shoot dry weight	(67)
Forage oats	-	Shoot biomass, 50% flowering	(68)
Buffel grass	-	Dry weight	(29)
Alfalfa	-	Shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, total fresh biomass, total dry biomass	(69)
Perennial ryegrass, cocksfoot, tall fescue, chicory	-	Leaf elongation rate, shoot dry weight, root dry weight	(37)
Perennial ryegrass	-	Plant height, leaf width, root activity	(70)
<i>Chloris gayana</i> , <i>Setaria anceps</i>	-	Number of tillers, number of leaves, fresh weight, dry biomass, plant height	(71)
<i>Setaria viridis</i>	-	Root dry mass, shoot dry mass, leaf size	(72)
<i>Agrostis scabra</i> , <i>A. stolonifera</i>	-	Root viability	(73)
Tall fescue, perennial ryegrass	-	Root dry weight, root viability	(36)

Tall fescue	-	Growth rate	(74)
Tall fescue	-	Plant height, dry weight	(75)

membrane degradation, heat stress lowers relative water content, chlorophyll, and photosynthetic efficiency while raising electrolyte leakage, evapotranspiration, and canopy temperature, reducing fodder quality. In contrast, *Setaria viridis* showed increased tissue nitrogen and C:N ratio under heat stress (72), a concentration effect in which heat suppresses biomass accumulation and carbon fixation more than nitrogen uptake.

3. Response of plants to abiotic stresses

Abiotic stress triggers a variety of physiological and biochemical reactions in plants. This section addresses how physiological traits and metabolism are altered to combat abiotic stressors. Figure 1. illustrates the responses of fodder crops to abiotic stresses.

3.1. Response of plants to drought stress

Proline accumulates under drought stress as an osmoprotectant that maintains cellular turgidity, in *Cynodon dactylon* and *Lolium perenne* (41), *Pennisetum glaucum* (39), *Agropyron desertorum* (40), alfalfa (23), (32), *Cenchrus ciliaris* and *Cyperus arenarius* (33), fodder maize

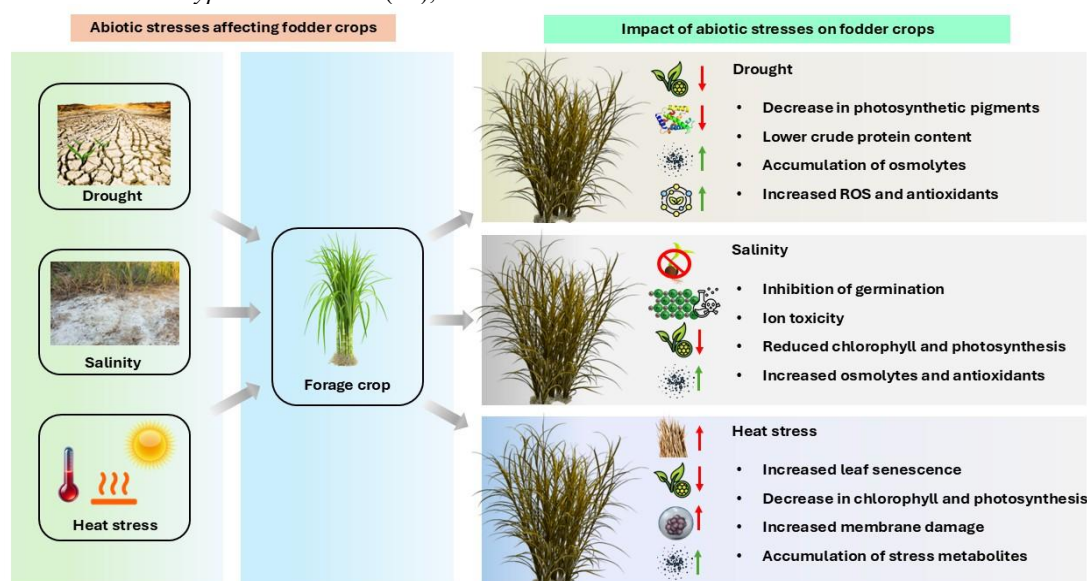


Figure 1. Responses of fodder crops to abiotic stresses.

(24), *Elymus elongatum* (42), berseem clover (27), and fodder sorghum (19). Flavonoid and soluble sugar or protein content rise alongside proline in *Festulolium* (30), alfalfa (44), rye (76) and *Festuca arundinacea* (77), providing further antioxidant and osmotic protection, though lucerne showed lower soluble sugars, glutathione, catalase, and peroxidase despite raised SOD and MDA (38).

Malondialdehyde (MDA), a marker of lipid peroxidation from drought-induced ROS, increased alongside antioxidant enzymes such as catalase, superoxide dismutase (SOD), peroxidase, and ascorbate peroxidase (APX) in most stressed species, including alfalfa (23), *Agropyron desertorum* (40), *Cynodon dactylon* (41), buffel grass (29), and fodder sorghum (19), indicating an active but sometimes overwhelmed antioxidant defense; in Kentucky blue grass, however, SOD and catalase activity fell as MDA increased (47), and prolonged severe drought reduced SOD activity in *Agropyron desertorum* after an initial increase (40). Enzymatic antioxidants scavenge ROS such as superoxide, hydrogen peroxide, and hydroxyl radicals that otherwise damage the photosystem and cellular organelles, but their activity declines in genotypes unable to sustain defense under severe stress.

Protein and water-soluble carbohydrate content, along with phenolic compounds that scavenge free radicals, increased in water-stressed forage grasses (30), (41) and improved fibre digestibility, while *Pennisetum glaucum* (39) and *Cenchrus ciliaris* and *Cyperus arenarius* (33) accumulated more amino acids, ascorbic acid, and phenolics as proteins were broken down for osmotic adjustment. These soluble proteins and carbohydrates act as osmoprotectants that regulate osmotic potential and cellular turgidity under drought. These responses are summarized in Table 4.

3.2. Response of plants to salinity stress

Proline accumulates under salinity stress in most species tested, including Marvel grass (62), *Euphorbia lathyris* (51), alfalfa (56), and *Avena* species (60), serving as an osmolyte that helps regulate osmotic potential, induce stomatal closure, and limit water loss. Reactive oxygen

Table 4. Physiological and biochemical responses of fodder crops to drought stress

Crops	Increased	Decreased	References
<i>Agropyron desertorum</i>	Malondialdehyde, proline content, CAT, POD, APX, GPX	Chlorophyll content	(40)

<i>Poa pratensis</i>	Proline content, carbohydrates, catalase, superoxide dismutase	Chlorophyll content, ascorbate peroxidase	(41)
<i>Cenchrus ciliaris</i> , <i>C. arenarius</i>	Glycine betaine, proline, total soluble sugars, ascorbic acid, phenolic content, total soluble protein, malondialdehyde, hydrogen peroxide, SOD, catalase, peroxidase	Chlorophyll a, chlorophyll b, total chlorophyll	(33)
Alfalfa	Chlorophyll b, total chlorophyll, proline, soluble proteins, soluble sugars, malondialdehyde, superoxide, hydrogen peroxide, hydroxyl radicals, SOD, peroxidase, catalase, reduced ascorbic acid, glutathione peroxidase	Chlorophyll a, leaf water potential, relative water content	(23)
<i>Festulolium braunii</i>	-	Photosynthetic rate, transpiration rate, stomatal conductance	(22)
Tall fescue	Canopy temperature	Leaf relative water content, photosynthetic rate, evapotranspiration rate, transpiration rate, stomatal conductance	(34)
Fodder maize	Proline, soluble carbohydrates, MDA, CAT activity	Chlorophyll a, chlorophyll b, total chlorophyll, carotenoids	(24)
Rye	Soluble protein, soluble sugar, MDA	Relative water content, chlorophyll content	(76)
Napier grass	-	Fv/F0, Fv/Fm	(26)
<i>Poa pratensis</i> , <i>Lolium perenne</i>	Cell membrane stability, cell wall elasticity	Leaf chlorophyll content, Fv/Fm ratio, leaf relative water content, carbohydrates, osmotic potential, turgid weight to dry weight ratio	(43)
Berseem clover	Proline content	Leaf water potential, osmotic potential, relative water content	(27)
Berseem	-	Canopy temperature depression, transpiration rate	(28)
Lucerne	Proline, glycine betaine, APX, SOD, malondialdehyde	Relative leaf water content, total chlorophyll content, nitrate reductase activity	(44)
Lucerne	Chlorophyll stability index, relative water content, superoxide dismutase, MDA content	Photosynthetic rate, soluble sugars, glutathione, catalase, peroxidase	(38)
<i>Urochloa</i> grass	Non-photochemical quenching	Relative water content, relative chlorophyll content	(45)
Fodder sorghum	-	Alpha amylase activity, relative water content	(16)
Fodder sorghum	Number of stomata, malondialdehyde, peroxidase, APX, catalase, SOD, proline	Relative water content, membrane stability index, SPAD values	(19)
Perennial ryegrass, cocksfoot, tall fescue, chicory	Canopy temperature depression	Fv/Fm ratio, cell membrane permeability, relative leaf water content	(37)
<i>Poa labillardierei</i> , <i>Austrostipa ramosissima</i> , <i>Microlaena stipoides</i> , <i>Themeda triandra</i> , <i>Imperata cylindrica</i> , <i>Eragrostis elongata</i>	Leaf senescence	Leaf water content, photosynthetic efficiency	(46)

Tall fescue, perennial ryegrass	-	Soil water content, relative leaf water content, photosynthetic rate, chlorophyll fluorescence (Fv/Fm), cell membrane stability, evapotranspiration rate	(36)
<i>Elymus elongatum</i>	Proline content	Relative water content	(42)
Kentucky blue grass	Electrolyte leakage, malondialdehyde content	Relative water content, SOD, catalase activity	(47)
<i>Festulolium braunii</i> , <i>Festuca arundinacea</i> , <i>Lolium perenne</i>	-	Chlorophyll content, flavonol content, photosynthetic rate, transpiration rate, stomatal conductance, water use efficiency, relative water content	(35)
Tall fescue, Italian ryegrass, perennial ryegrass, meadow fescue	Water use efficiency, leaf surface temperature, proline content, flavonoids	Photosynthetic rate, transpiration rate, stomatal conductance, CO ₂ accumulation	(30)
Alfalfa	Proline content, soluble sugars, malondialdehyde content	Relative water content, osmotic potential	(32)
Pearl millet (<i>Pennisetum glaucum</i>)	Proline content, amino acid	-	(39)
<i>Agropyron cristatum</i> , <i>A. intermedium</i> , <i>Festuca ovina</i> , <i>Festuca arundinacea</i> , <i>Cynodon dactylon</i> , <i>Bromus inermis</i> , <i>B. confinis</i>	Osmotic potential, total soluble carbohydrates, sucrose content, proline content, H ₂ O ₂ content, glutathione reductase activity, total ascorbate content, total soluble protein	-	(77)
Buffel grass	MDA content, total reducing power (FRAP), CAT, SOD	-	(29)

species such as superoxide, hydrogen peroxide, and MDA also rise, as in *Euphorbia lathyris* (51) and alfalfa (56), causing oxidative damage to DNA, proteins, and chloroplasts. In response, antioxidant enzyme activity, particularly SOD, catalase, peroxidase, and APX, increases in alfalfa (54), (57), switchgrass (78), *Avena* species (60), and *Euphorbia lathyris* (51) to protect proteins, lipids, and DNA from ROS, with SOD and catalase more active in roots and peroxidase and APX more active in leaves for detoxifying hydrogen peroxide. Chlorophyll a is preferentially degraded by salinity-induced ROS, reflected in leaf yellowing in hybrid Giant Napier (63), while carotenoids act as antioxidants and can increase under stress. Soluble protein and carbohydrate content also increased under salinity in *Euphorbia lathyris* (51) and alfalfa (54), acting as osmoprotectants that stabilise membranes, scavenge ROS, and prevent cellular dehydration. These responses are summarized in Table 5.

Table 5. Physiological and biochemical responses of fodder crops to salinity stress

Crops	Increased	Decreased	References
Pearl millet x Napier hybrid	Na concentration, K ⁺ :Na ⁺ ratio	Chlorophyll <i>a</i> , chlorophyll <i>b</i> , total chlorophyll, carotenoid, chlorophyll stability index, carotenoid stability index, leaf relative water content, membrane stability index, K concentration	(61)
Marvel Grass	Na accumulation, proline content	Total chlorophyll, photosynthetic rate, transpiration rate, stomatal conductance, K accumulation	(62)
Napier grass hybrid	Stomatal resistance	Chlorophyll content, transpiration rate, stomatal conductance	(65)
<i>Avena</i> species	SPAD value, Na concentration, malondialdehyde, proline content, SOD, CAT, APX	Membrane stability index, relative water content, K concentration	(60)
Rhodes grass	-	SPAD value	(53)
Alfalfa	Proline content, hydrogen peroxide	Membrane stability index, relative water content	(56)
Italian ryegrass	Na accumulation, Na/K ratio	K accumulation	(49)

<i>Euphorbia lathyris</i>	Na concentration, proline, superoxide, hydrogen peroxide, malondialdehyde, SOD, POX, CAT, ascorbic acid, soluble proteins, soluble sugars	K ⁺ , Ca ²⁺ and Mg ²⁺ concentration	(51)
Alfalfa	SOD, POD, APX, CAT activity, malondialdehyde, reducing sugars	-	(54)
Alfalfa	Malondialdehyde, POX, CAT, APX activities	-	(57)
Alfalfa	SOD, CAT, POD, APX activities	Glutathione reductase	(58)

3.3. Responses of plants to heat stress

Heat stress raises MDA, soluble sugar, and soluble protein content alongside antioxidant enzyme activity (MDA, FRAP, CAT, SOD) in tolerant buffel grass (29) and lucerne genotypes (69), though SOD and catalase activity fell in Kentucky blue grass (47) and became inactivated in tall fescue (75) despite rising MDA, with citric acid improving tall fescue's heat tolerance. In *Setaria viridis*, heat stress raised PEPC activity, malate, sugars, proline, and abscisic acid while lowering aspartate, glutamate, and starch content (72), reflecting a shift toward carbon-conserving metabolism, osmolyte accumulation, and ABA-driven stomatal closure. Increased leaf soluble protein and heat-induced proteins in Kentucky bluegrass (47), bent grass, and perennial ryegrasses (66) reflect activation of heat shock proteins and chaperones that stabilise cellular proteins and membranes, whereas most heat-responsive proteins were downregulated in *Agrostis scabra* and *A. stolonifera* (73). Heat stress also lowered transcripts of carbonic anhydrase, PEPC, NADP-MDH, RBCL, RBCS, and PPK in *Setaria viridis* (72), suppressing CO₂-concentrating mechanisms and Calvin-cycle activity. Overall, heat stress raises ROS and lipid peroxidation, which tolerant genotypes counter more effectively than susceptible ones. These responses are summarized in Table 6.

Table 6. Physiological and biochemical responses of fodder crops to heat stress

Crops	Increased	Decreased	References
Fodder maize	Starch content, protein content, crude fibre content, metabolizable energy	-	(67)
Forage oat	-	Chlorophyll SPAD reading	(68)
Alfalfa	Chlorophyll fluorescence, malondialdehyde content, soluble sugar content, soluble protein	Leaf relative water content, chlorophyll content	(69)
Perennial ryegrass, cocksfoot, tall fescue, chicory	Canopy temperature depression	Fv/Fm ratio, cell membrane permeability, relative leaf water content	(37)
Perennial ryegrass	Electrolyte leakage	Water use efficiency, photosynthetic rate, chlorophyll content, relative water content, Fv/Fm ratio	(70)
<i>Poa labillardierei</i> , <i>Austrostipa ramosissima</i> , <i>Microlaena stipoides</i> , <i>Themeda triandra</i> , <i>Imperata cylindrica</i> , <i>Eragrostis elongata</i>	Leaf senescence	Leaf water content, photosynthetic efficiency	(46)
Kentucky bluegrass, bent grass, perennial ryegrasses	Leaf soluble protein content, heat induced proteins	Chlorophyll content	(66)
<i>Chloris gayana</i> , <i>Setaria anceps</i>	-	Relative water content, chlorophyll a, chlorophyll b, total chlorophyll content	(71)
Tall fescue, perennial rye grass	-	Soil water content, relative leaf water content, photosynthetic rate, chlorophyll fluorescence (Fv/Fm), cell membrane stability, evapotranspiration rate	(36)

Kentucky blue grass	Electrolyte leakage, malondialdehyde content	Relative water content, SOD and catalase activities	(47)
Tall fescue	-	Chlorophyll content, evapotranspiration rate	(74)
Tall fescue	-	Chlorophyll content, Fv/Fm ratio, SOD, peroxidase, catalase activities	(75)
Buffel grass	Malondialdehyde (MDA) content, total reducing power (FRAP), CAT, SOD	-	(29)
<i>Setaria viridis</i>	PEPC activity, malate content, aspartate aminotransferase, PEPC:Rubisco ratio, leaf sucrose, glucose, fructose, trehalose, raffinose, proline, soluble sugars, soluble alcohols, leaf protein contents, abscisic acid content	Aspartate, glutamate, leaf starch content, CA (carbonic anhydrase), PEPC, NADP-MDH, RBCL, RBCS, PPK	(72)

4. Strategies to improve pearl millet x napier hybrids for abiotic stress tolerance

To produce fodder under varied environmental and climatic conditions and increase animal output year-round, genetic modification of fodder crops for abiotic stress tolerance is crucial, particularly to improve output and quality under unfavourable conditions and boost productivity and cultivated area. Figure 2. illustrates the strategies to improve abiotic stress tolerance in fodder crops.

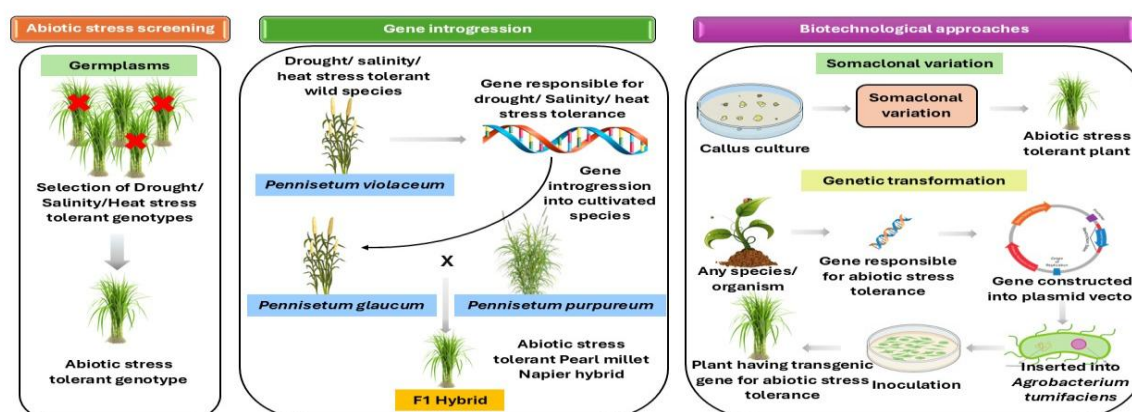


Figure 2. Strategies to improve abiotic stress tolerance.

4.1. Selection for abiotic stress tolerance

Screening germplasm, landraces, advanced lines, and wild relatives under abiotic stress identifies genotypes suited to adverse conditions, with indirect selection possible via traits correlated with stress tolerance. Under drought, grass quality in *Agropyron desertorum* correlated positively with leaf wilting and chlorophyll content (40). Dry matter yield correlated with net photosynthesis, dry matter content, and water-use efficiency in *Festuca*, *Lolium*, and *Festulolium* (30), and in *Cenchrus ciliaris* and *Cyperus arenarius*, potassium and calcium content correlated with growth and photosynthesis while sodium correlated with oxidative stress markers (33). These associations suggest that selecting for shoot and root fresh weight, biomass, water-use efficiency, chlorophyll content, and nutrient status can identify drought-tolerant genotypes.

Under salinity, shoot dry weight correlated positively with root dry weight, root-shoot ratio, relative water content, membrane stability index, chlorophyll *b*, total chlorophyll, carotenoid, and potassium concentration in *Cenchrus* and *Pennisetum* hybrids, with membrane stability and the $K^+:Na^+$ ratio also closely linked (61). Proline content correlated with root Na^+ content in *Dichanthium annulatum* (62). Selecting for these traits, shoot and root biomass, relative water content, membrane stability, pigment and proline content, favourable ion ratios, and gas-exchange parameters offers an indirect route to improving salinity and abiotic stress tolerance in Pearl millet x Napier hybrids.

4.2. Introgression of gene responsible for abiotic stress tolerance

Introgressing abiotic stress-tolerance genes from wild and related species through backcross and pedigree breeding, aided by molecular markers, is among the most effective ways to improve fodder crops' stress tolerance. Drought-tolerant genes were introduced into *Lolium multiflorum* from *Festuca glaucescens* using the STS marker Fg71763 and GISH analysis (79). In pearl millet, SSR markers PSMP2066, PSMP2077, PSMP2059, and

PSMP2078 were used to introgress drought-tolerant QTLs from 863B into HBL 11, and backcross progenies carrying these QTLs showed improved plant height, tillering, leaf length, relative water content, and chlorophyll fluorescence under drought (80). Introgression of biotic and abiotic resistance genes from *Pennisetum violaceum* into *P. glaucum* produced roughly 93 lines with good dry fodder yield under drought (81), showing wild relatives to be a valuable source of drought-tolerance genes transferable to Pearl millet x Napier hybrids. For salinity, QTLs introgressed from the PRLT2/89-33 accession into six pearl millet genotypes produced near-isogenic lines with higher germination, biomass, and lower sodium and potassium accumulation than sensitive parents (82), suggesting similar transfer into Napier grass could develop salinity-tolerant Pearl millet x Napier hybrids.

4.3. Biotechnological approaches to improve abiotic stress tolerance

In vitro mutagenesis using gamma rays and NaCl selection produced salt-tolerant Ruzi grass mutants with narrow leaves, tall stems, multiple clumps, and rapid growth (83), showing that induced mutation can generate abiotic-stress-tolerant variation applicable to Pearl millet x Napier hybrids. Somaclonal variation arising in vitro, through chromosomal, gene-sequence, methylation, or transposable-element changes, similarly offers useful diversity: Napier grass genotypes able to form callus under high PEG concentrations (simulated drought) were more drought-tolerant, likely reflecting such variation (84).

Agrobacterium-mediated transformation of tobacco with the *Pennisetum purpureum* *PpCCoAOMT* gene reduced drought-induced declines in leaf area, transpiration, and stomatal traits while increasing proline, relative water content, and SOD, POD, CAT, and APX activity relative to untransformed plants (85), indicating *PpCCoAOMT* drives morphological and physiological changes that improve drought tolerance. Similarly, *Arabidopsis thaliana* transformed with the *Pennisetum glaucum* *PgDREB2A* gene showed unaffected root and shoot growth under drought and salinity, longer roots, cold tolerance, and retained greenness under heat, along with lower MDA and higher relative water content, proline, and chlorophyll under drought and full survival under salt stress (86), pointing to *PgDREB2A* as a target for engineering multi-stress tolerance. In heat-stressed transgenic lucerne overexpressing miR156, plants remained greener with higher water potential, antioxidant activity, anthocyanin, and chlorophyll content than controls, alongside altered expression of MYB53, CCD1, NCED3, HSP17, and LOB, and SPL13i genotypes showed enhanced antioxidant capacity, water potential, and stomatal conductance (87). Somaclonal variation, mutation breeding, and genetic engineering, including *Agrobacterium*-mediated transfer of genes such as *PgDREB2A* and *PpCCoAOMT*, thus offer complementary routes to abiotic-stress-tolerant Pearl millet x Napier hybrids, provided transgenic plants are confirmed safe for livestock feeding. Key genes for abiotic stress tolerance are listed in Table 7.

Table 7. Genes involved in abiotic stress tolerance

Genes or QTL	Crop species	Stress	Role	Reference
LG2 and LG5 QTLs	Pearl millet	Drought stress	Improves plant height, number of tillers, leaf length, relative water content, chlorophyll fluorescence	(80)
<i>PpCCoAOMT</i>	<i>Pennisetum purpureum</i>	Drought stress	Increases the concentration of proline, relative leaf water content, the activity of SOD, POD, CAT and APX	(85)
<i>PgDREB2A</i>	<i>Pennisetum glaucum</i>	Salinity stress	Improves root and shoot growth; increases relative water content, proline content and total chlorophyll content; decreases malondialdehyde concentration	(86)
<i>miR156</i>	Alfalfa	Heat stress	High water potential, increased antioxidant activity, increased anthocyanin content, high level of chlorophyll a, b and total chlorophyll content	(87)

5. CONCLUSION

Drought, salinity and heat stresses substantially alter plant morphology, physiology, and biochemistry, lowering photosynthesis, nutrient and water uptake, and increasing ion toxicity and membrane and photosystem damage, reducing fodder supply and quality year-round. In response, plants employ osmotic adjustment, proline and soluble carbohydrate and protein accumulation, enhanced antioxidant enzyme activity, and morphological modifications. Understanding these impacts and responses lays the foundation for developing abiotic stress-tolerant pearl millet x Napier hybrid grass.

Genetic improvement of Pearl millet x Napier hybrid grass for abiotic stress tolerance can draw on selection, gene introgression, molecular breeding, tissue culture, and genetic engineering. Most stress-tolerance genes reside in wild accessions and related genera and species; those from closely related, easily crossable species such as *Pennisetum purpureum* and *P. violaceum* offer a particularly accessible source for transfer into Pearl millet x Napier hybrids. Applying these techniques together can help produce climate-resilient Pearl millet x Napier hybrid grass for sustained, year-round fodder production.

ACKNOWLEDGEMENTS

I would like to express my deepest gratitude to Tamil Nadu Agricultural University for providing the academic environment and institutional support necessary for the completion of this review. No funding was provided.

Author Contributions

MV conceptualized the review framework and prepared the original draft of the manuscript. TE provided overall guidance, refined the research theme, and critically reviewed and approved the final manuscript. RP assisted in structuring the review, improving technical content, and revising the manuscript. KC and AS contributed expert insights and critically evaluated the manuscript. MV contributed to literature compilation, conceptualization, review & editing. All authors reviewed and approved the final version of the manuscript before submission.

Data availability

All information used in this study was obtained from previously published sources, including peer-reviewed journal articles, and established academic databases such as Scopus and Web of Science. This study did not generate or analyze any new primary datasets.

Compliance with ethical standards

Conflict of interest: The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

REFERENCES

1. Devendra C, Thomas D. Crop–animal systems in Asia: importance of livestock and characterisation of agro-ecological zones. *Agricultural systems*. 2002;71(1-2):5-15.[https://doi.org/DOI:10.1016/S0308-521X\(01\)00032-4](https://doi.org/DOI:10.1016/S0308-521X(01)00032-4)
2. Ugli OQQ. The Role and Importance of Livestock Sectors in Agriculture. *World Economics and Finance Bulletin*. 2021;4:16-9
3. Upton M. The role of livestock in economic development and poverty reduction. 2004
4. Kumar S, Singh P, Devi U, Yathish K, Saujanya PL, Kumar R, et al. An overview of the current fodder scenario and the potential for improving fodder productivity through genetic interventions in India. *Animal Nutrition and Feed Technology*. 2023;23(3):631-44
5. Koli P, Bhardwaj NR. Status and use of pesticides in forage crops in India. *Journal of pesticide science*. 2018;43(4):225-32.<https://doi.org/10.1584/jpestics.d18-004>
6. Kapoor R, Singh TP, Khosla G. Biotechnological interventions in forage crops-a review. *International Journal of Current Microbiology and Applied Sciences*. 2018;7(7):1229-40.<https://doi.org/10.20546/ijcmas.2018.707.148>
7. Reddy AM, Kumari CR, Reddy BS, Reddy BR. Productivity and quality of fodder crops under late-sown conditions in semi-arid tropics of India. *Indian Journal of Agricultural Research*. 2022(6):660-5.<https://doi.org/10.18805/ijare.a-6005>
8. Hindoriya PS, Kumar R, Kumar S, Bhattacharjee S. NAPIER BAJRA HYBRID: AN IDEAL CROP FOR YEAR ROUND FODDER PRODUCTION. 2021
9. Mohan S, Dar EA, Singh M. Fodder quality of teosinte fodder as influenced by nitrogen, phosphorus and zinc application. *Int J Pure Appl Biosci*. 2017;5:596-604.<https://doi.org/10.18782/2320-7051.2557>
10. Yanqing W, Jiao L, Lu Z, Hao W, Yiming Z, Irshad A, et al. Abiotic stress responses in crop plants: A multi-scale approach. *Journal of Integrative Agriculture*. 2026;25(1):1-15.<https://doi.org/10.1016/j.jia.2024.09.003>
11. Mishra UN, Chauhan J, Singhal RK, Anuragi H, Dey P, Lal D, et al. Abiotic stress responses in forage crops and grasses: the role of secondary metabolites and biotechnological interventions. *Frontiers in Plant Science*. 2025;16:1542519.<https://doi.org/10.3389/fpls.2025.1542519>
12. Verma P, Sharma TR, Srivastava PS, Abdin M, Bhatia S. Exploring genetic variability within lentil (*Lens culinaris* Medik.) and across related legumes using a newly developed set of microsatellite markers. *Molecular biology reports*. 2014;41(9):5607-25.<https://doi.org/10.1007/s11033-014-3431-z>
13. Ressaissi N, Sanchez-Ballesta MT, Badri M. Influence of drought and heat stresses in fodder legumes productivity and nutritive value. *Journal of Oasis Agriculture and Sustainable Development*. 2023;5(2):18-26.<https://doi.org/10.56027/joasd.092023>
14. Sultan MT, Mahmud U, Khan MZ. Addressing soil salinity for sustainable agriculture and food security: Innovations and challenges in coastal regions of Bangladesh. *Elsevier*; 2023. p. 100260.
15. Khan MZ, Azom MG, Sultan MT, Mandal S, Islam MA, Khatun R, et al. Amelioration of saline soil by the application of gypsum, calcium chloride, rice husk and cow dung. *Journal of Agricultural Chemistry and Environment*. 2019;8(2):78-91.<https://doi.org/10.4236/jacen.2019.82007>
16. Khokhar Y, Indu I, Ahmed S, Rana M, Singh M, Singh S, et al. Seedling growth responses of fodder sorghum [*Sorghum bicolor* (L.) Moench] genotypes under drought stress. *Discover Plants*. 2025;2(1):147.<https://doi.org/10.1007/s44372-025-00230-3>
17. Greeshma U, Thomas UC, Shalini PP, Gayathri G, Safeer M, Gopinath PP. Performance evaluation of different bajra napier hybrid varieties under various irrigation levels in southern Kerala. 2024
18. Arshad I, Ali W, Khan ZA, Bhayo WA. Effect of water stress on the growth and yield of Rhodes grass (*Chloris gayana* L. Kunth.). *PSM Biological Research*. 2016;1(2):58-61
19. Indu I, Khokhar Y, Singh M, Ahmed S, Rana M, Chand S, et al. Antioxidant Defense System Mediating Water Stress Tolerance through Improving Growth Attributes in Fodder Sorghum (*Sorghum bicolor* L. Moench). *Russian Journal of Plant Physiology*. 2025;72(4):99.<https://doi.org/10.1134/s1021443724609911>
20. Jahanzad E, Jorat M, Moghadam H, Sadeghpour A, Chaichi M-R, Dashtaki M. Response of a new and a commonly grown forage sorghum cultivar to limited irrigation and planting density. *Agricultural water management*. 2013;117:62-9.<https://doi.org/10.1016/j.agwat.2012.11.001>

21. Somegowda VK, Vemula A, Naravula J, Prasad G, Rayaprolu L, Rathore A, et al. Evaluation of fodder yield and fodder quality in sorghum and its interaction with grain yield under different water availability regimes. *Current Plant Biology*. 2021;25:100191.<https://doi.org/10.1016/j.cpb.2020.100191>
22. Mastalerczuk G, Borawska-Jarmulowicz B. Physiological and morphometric response of forage grass species and their biomass distribution depending on the term and frequency of water deficiency. *Agronomy*. 2021;11(12):2471.<https://doi.org/10.3390/agronomy11122471>
23. Zhang C, Shi S, Wang B, Zhao J. Physiological and biochemical changes in different drought-tolerant alfalfa (*Medicago sativa* L.) varieties under PEG-induced drought stress. *Acta Physiologiae Plantarum*. 2018;40(2):25.<https://doi.org/10.1007/s11738-017-2597-0>
24. Beigi A, Ghooshchi F, Moghaddam HT, Nasiri M, Kasraie P. Effect of methyl jasmonate and potassium and their combined action on drought tolerance of fodder corn. *Russian Journal of Plant Physiology*. 2023;70(5):102.<https://doi.org/10.1134/s1021443723600733>
25. Esmaily M, Dadashi MR, Feyzbakhsh MT, Kaboosi K, Sheikh F. Influence of deficit irrigation regimes on the quantitative and qualitative yield of forage maize hybrids. *Journal of Crop Health*. 2024;76(2):549-60.<https://doi.org/10.1007/s10343-024-00973-1>
26. Kang D-J, Tazoe H. Impacts of soil type and drought stress on growth and cesium accumulation in Napier grass. *Environmental Geochemistry and Health*. 2024;46(7):235.<https://doi.org/10.1007/s10653-024-02023-1>
27. Iannucci A, Rascio A, Russo M, Di Fonzo N, Martiniello P. Physiological responses to water stress following a conditioning period in berseem clover. *Plant and Soil*. 2000;223(1):219-29.<https://doi.org/10.1023/a:1004842927653>
28. Hussain MM, Rauf S, Paderewski J, UlHaq I, Sienkiewicz-Paderewska D, Monneveux P. Multitraits evaluation of Pakistani ecotypes of berseem clover (*Trifolium alexandrinum* L.) under full-irrigation and water restriction conditions. 2015
29. Tommasino E, Colomba EL, Carrizo M, Grunberg K, Quiroga M, Carloni E, et al. Individual and combined effects of drought and heat on antioxidant parameters and growth performance in Buffel grass (*Cenchrus ciliaris* L.) genotypes. *South African journal of botany*. 2018;119:104-11.<https://doi.org/10.1016/j.sajb.2018.08.026>
30. Fariaszewska A, Aper J, Van Huylenbroeck J, Baert J, De Riek J, Staniak M, et al. Mild drought stress-induced changes in yield, physiological processes and chemical composition in *Festuca*, *Lolium* and *Festulolium*. *Journal of agronomy and crop science*. 2017;203(2):103-16.<https://doi.org/10.1111/jac.12168>
31. Taleb MH, Majidi MM, Pirnajmedin F, Maibody SAMM. Plant functional trait responses to cope with drought in seven cool-season grasses. *Scientific reports*. 2023;13(1):5285.<https://doi.org/10.1038/s41598-023-31923-y>
32. Slama I, Tayachi S, Jdey A, Rouached A, Abdely C. Differential response to water deficit stress in alfalfa (*Medicago sativa*) cultivars: Growth, water relations, osmolyte accumulation and lipid peroxidation. *African Journal of Biotechnology*. 2011;10(72):16250-9.<https://doi.org/10.5897/AJB.9000149>
33. Ghafar MA, Akram NA, Saleem MH, Wang J, Wijaya L, Alyemeni MN. Ecotypic morphological and physio-biochemical responses of two differentially adapted forage grasses, *Cenchrus ciliaris* L. and *Cyperus arenarius* Retz. to drought stress. *Sustainability*. 2021;13(14):8069.<https://doi.org/10.3390/su13148069>
34. Sun J, Meyer W, Cross J, Huang B. Growth and physiological traits of canopy and root systems associated with drought resistance in tall fescue. *Crop Science*. 2013;53(2):575-84.<https://doi.org/10.2135/cropsci2012.05.0292>
35. Mastalerczuk G, Borawska-Jarmulowicz B, Darkalt A. Changes in the physiological and morphometric characteristics and biomass distribution of forage grasses growing under conditions of drought and silicon application. *Plants*. 2022;12(1):16.<https://doi.org/10.3390/plants12010016>
36. Jiang Y, Huang B. Physiological responses to heat stress alone or in combination with drought: A comparison between tall fescue and perennial ryegrass. *HortScience*. 2001;36(4):682-6.<https://doi.org/10.21273/hortsci.36.4.682>
37. Perera RS, Cullen BR, Eckard RJ. Growth and physiological responses of temperate pasture species to consecutive heat and drought stresses. *Plants*. 2019;8(7):227.<https://doi.org/10.3390/plants8070227>
38. Antony E, Kumar S, Sridhar K, Karthigeyan S, Kulkarni N, Doddamani M. Physiological adaptations of lucerne under a limited irrigation system. *Range Management and Agroforestry*. 2024;45(01):65-71.<https://doi.org/10.59515/rma.2024.v45.i1.09>
39. Suganthi S, Sivagami K, Maheswari C, Manivannan P. Effect of drought stress on biochemical contents and proline metabolizing enzymes of *Pennisetum glaucum* L. *Innovations in Agriculture*. 2021;4:e32832.<https://doi.org/10.25081/ia.2021.v4.2797>
40. Tatari M, Ghazvini RF, Etemadi N, Ahadi AM, Mousavi A. Analysis of antioxidant enzymes activity, lipid peroxidation and proline content of *Agropyron desertorum* under drought stress. *Biology and Environment*. 2012;3(1):9-24
41. Seyedmohammadi SA, Jafari AA, Khodarahmpour Z, Sadat S. The Effect of Water Deficit Conditions on the Physiological Responses of Seven Amenity Grass Species. *Journal of Crop Nutrition Science*. 2024;10(3).<https://doi.org/10.71874/jcns.2024.16576>
42. Gazanchian A, Hajheidari M, Sima NK, Salekdeh GH. Proteome response of *Elymus elongatum* to severe water stress and recovery. *Journal of Experimental Botany*. 2007;58(2):291-300.<https://doi.org/10.1093/jxb/erl226>

43. Chai Q, Jin F, Merewitz E, Huang B. Growth and physiological traits associated with drought survival and post-drought recovery in perennial turfgrass species. *Journal of the American Society for Horticultural Science*. 2010;135(2):125-33.<https://doi.org/10.21273/jashs.135.2.125>
44. Diware R, Damame S. Biochemical evaluation of lucerne (*Medicago sativa* L.) cultivars under water stress condition. *Journal of Pharmacognosy and Phytochemistry*. 2019;8(6):2042-5
45. Ochola CA, Ngugi MP, Nyaboga EN, Njarui DM. Morpho-physiological and yield traits for selection of drought tolerant *Urochloa* grass ecotypes. *AoB Plants*. 2024;16(3):plae034.<https://doi.org/10.1093/aobpla/plae034>
46. Davies M, Ecroyd H, Robinson SA, French K. Stress in native grasses under ecologically relevant heat waves. *PLoS One*. 2018;13(10):e0204906.<https://doi.org/10.1371/journal.pone.0204906>
47. Liu J, Xie X, Du J, Sun J, Bai X. Effects of simultaneous drought and heat stress on Kentucky bluegrass. *Scientia Horticulturae*. 2008;115(2):190-5.<https://doi.org/10.1016/j.scienta.2007.08.003>
48. Shen YY, Li Y, Yan SG. Effects of salinity on germination of six salt-tolerant forage species and their recovery from saline conditions. *New Zealand Journal of Agricultural Research*. 2003;46(3):263-9.<https://doi.org/10.1080/00288233.2003.9513552>
49. Xie Y, Liu X, Amee M, Yu H, Huang Y, Li X, et al. Evaluation of salt tolerance in Italian ryegrass at different developmental stages. *Agronomy*. 2021;11(8):1487.<https://doi.org/10.3390/agronomy11081487>
50. Akhtar P, Hussain F. Salinity tolerance of three range grasses at germination and early growth stages. *Pak J Bot*. 2008;40(6):2437-41
51. Yang J, Cao Y, Yang Z, Zhang W, Sun L, Lu C. Morphological, physiological and biochemical responses of biofuel plant *Euphorbia lathyris* to salt stress. *Acta Agriculturae Scandinavica, Section B–Soil & Plant Science*. 2013;63(4):330-40.<https://doi.org/10.1080/09064710.2013.778327>
52. Abdolrhman I, Almathnani A, Abobaker A, Saber A, Alsadi S. Evaluation of salinity effect on growth and productivity of Rhodes grass (*Chloris gayana*). 2021
53. Daba AW, Qureshi AS, Nisaren BN. Evaluation of some Rhodes grass (*Chloris gayana*) genotypes for their salt tolerance, biomass yield and nutrient composition. *Applied Sciences*. 2019;9(1):143.<https://doi.org/10.3390/app9010143>
54. Babakhani B, Khavari-Nejad R, Fahimi H, Saadatmand S. Biochemical responses of Alfalfa (*Medicago sativa* L.) cultivars subjected to NaCl salinity stress. *African Journal of Biotechnology*. 2011;10(55):11433-41.<https://doi.org/10.5897/AJB11.594>
55. Alam M, Sarker N, Nasiruddin K, Shohael A. Salinity stress on morphological and nutritional quality of Napier cultivars under hydroponic condition. *Bangladesh Journal of Animal Science*. 2017;46(2):102-8.<https://doi.org/10.3329/bjas.v46i2.34438>
56. Chaparzadeh N, Mehrnejad F. Oxidative markers in five Iranian alfalfa (*Medicago sativa* L.) cultivars under salinity stress. 2013
57. Ashrafi E, Razmjoo J, Zahedi M, Pessarakli M. Screening alfalfa for salt tolerance based on lipid peroxidation and antioxidant enzymes. *Agronomy Journal*. 2015;107(1):167-73.<https://doi.org/10.2134/agronj14.0248>
58. Wang X, Wei Z, Liu D, Zhao G. Effects of NaCl and silicon on activities of antioxidative enzymes in roots, shoots and leaves of alfalfa. *African Journal of Biotechnology*. 2011;10(4):545
59. Azeem A, Wenxuan M, Ali R, Abbas A, Hussain N, Kazmi AH, et al. Evaluating salt tolerance in fodder crops: A field experiment in the dry land. *Open Agriculture*. 2024;9(1):20220307.<https://doi.org/10.1515/opag-2022-0307>
60. Ahmed S, Priyadarshini P, Indu I, Rana M, Yadav G, Rai A, et al. Effect of salinity stress on growth, physio-biochemical and ion homeostasis and activation of antioxidant defense responses in *Avena* spp. *Cereal Research Communications*. 2025;53(4):2237-56.<https://doi.org/10.1007/s42976-025-00648-1>
61. Dheeravathu SN, Singh K, Ramteke PW, Dikshit N, Prasad M, Deb D, et al. Physiological response of Bajra-Napier hybrids and Tri-specific Hybrid to salinity stress. *Tropical Grasslands-Forrajes Tropicales*. 2021;9(3):337-47.[https://doi.org/10.17138/tgft\(9\)337-347](https://doi.org/10.17138/tgft(9)337-347)
62. Kumar A, Kumar A, Kumar P, Lata C, Kumar S. Effect of individual and interactive alkalinity and salinity on physiological, biochemical and nutritional traits of Marvel grass. *Indian J Exp Biol*. 2018;56(8):573-81
63. Ma C, Naidu R, Liu F, Lin C, Ming H. Influence of hybrid giant Napier grass on salt and nutrient distributions with depth in a saline soil. *Biodegradation*. 2012;23(6):907-16.<https://doi.org/10.1007/s10532-012-9583-4>
64. Rahman M, Ishii Y, Niimi M, Kawamura O. Effect of salinity stress on dry matter yield and oxalate content in napiergrass (*Pennisetum purpureum* Schumach). *Asian-Australasian Journal of Animal Sciences*. 2008;21(11):1599-603.<https://doi.org/10.5713/ajas.2008.80217>
65. Maksup S, Sengsai S, Laosuntisuk K, Asayot J, Pongprayoon W. Physiological responses and the expression of cellulose and lignin associated genes in Napier grass hybrids exposed to salt stress. *Acta Physiologiae Plantarum*. 2020;42(7):109.<https://doi.org/10.1007/s11738-020-03092-2>
66. Zhou S, Abaraha A. Response to heat stress in warm season and cool season turf grass cultivars. *Sci Res Essays*. 2007;2(4):95-100
67. Mahmood S, Wahid A, Javed F, Basra SM. Heat stress effects on forage quality characteristics of maize (*Zea mays*) cultivars. *Int J Agric Biol*. 2010;12:701-6

68. Dheeravathu SN, Singh P, Srinivasan R, Yadav VK. Open Top Chamber: An innovative screening technique for temperature stress tolerance in forage oat (*Avena sativa*). *Forage Res.* 2022;47:513-6.<https://doi.org/10.59515/rma.2023.v44.i1.07>
69. Wassie M, Zhang W, Zhang Q, Ji K, Chen L. Effect of heat stress on growth and physiological traits of alfalfa (*Medicago sativa* L.) and a comprehensive evaluation for heat tolerance. *Agronomy.* 2019;9(10):597.<https://doi.org/10.3390/agronomy9100597>
70. Zhang J, Li H, Jiang Y, Li H, Zhang Z, Xu Z, et al. Natural variation of physiological traits, molecular markers, and chlorophyll catabolic genes associated with heat tolerance in perennial ryegrass accessions. *BMC plant biology.* 2020;20(1):520.<https://doi.org/10.1186/s12870-020-02695-8>
71. Haider T, Gulzar S, Malik SU, Saleem A, Hasnain Z, Hussain N, et al. Effect of Heat and Drought Stress on the Growth of Selected Grass Species of Pothohar Region. *Sarhad Journal of Agriculture.* 2025;41(1).<https://doi.org/10.17582/journal.sja/2025/41.1.32.41>
72. Zhang P, Sharwood RE, Carroll A, Estavillo GM, von Caemmerer S, Furbank RT. Systems analysis of long-term heat stress responses in the C4 grass *Setaria viridis*. *The Plant Cell.* 2025;37(4):koaf005.<https://doi.org/10.1093/plcell/koaf005>
73. Xu C, Huang B. Root proteomic responses to heat stress in two *Agrostis* grass species contrasting in heat tolerance. *Journal of Experimental Botany.* 2008;59(15):4183-94.<https://doi.org/10.1093/jxb/ern258>
74. Sun X, Du Z, Ren J, Amombo E, Hu T, Fu J. Association of SSR markers with functional traits from heat stress in diverse tall fescue accessions. *BMC Plant Biology.* 2015;15(1):116.<https://doi.org/10.1186/s12870-015-0494-5>
75. Hu L, Zhang Z, Xiang Z, Yang Z. Exogenous application of citric acid ameliorates the adverse effect of heat stress in tall fescue (*Lolium arundinaceum*). *Frontiers in plant science.* 2016;7:179.<https://doi.org/10.1186/s12870-015-0494-5>
76. Bao G, Pan X, Yan B, Chang Y, Tang W, Qu Y, et al. Resistance of Rye Seedlings to Drought and Freeze-Thaw Stress. *Polish Journal of Environmental Studies.* 2022;31(2).<https://doi.org/10.15244/pjoes/142608>
77. Khoshkholghsima NA, Rohollahi I. Evaluating biochemical response of some selected perennial grasses under drought stress in Iran. *Horticulture, Environment, and Biotechnology.* 2015;56(3):383-90.<https://doi.org/10.1007/s13580-015-0010-8>
78. Jin J, Niu J, Guo T, Zhou R, Sun LZ. The effect of drought on physiological responses of forage plants to salt stresses depends on occurring time. *Acta Physiologiae Plantarum.* 2020;42(6):91.<https://doi.org/10.1007/s11738-020-03083-3>
79. Humphreys J, Harper JA, Armstead IP, Humphreys MW. Introgression-mapping of genes for drought resistance transferred from *Festuca arundinacea* var. *glaucescens* into *Lolium multiflorum*. *Theoretical and Applied Genetics.* 2005;110(3):579-87.<https://doi.org/10.1007/s00122-004-1879-2>
80. Jangra S, Rani A, Yadav RC, Yadav NR, Yadav D. Introgression of terminal drought stress tolerance in advance lines of popular pearl millet hybrid through molecular breeding. *Plant Physiology Reports.* 2019;24(3):359-69.<https://doi.org/10.1007/s40502-019-00464-w>
81. Sharma S, Sharma R, Pujar M, Yadav D, Yadav Y, Rathore A, et al. Use of wild *Pennisetum* species for improving biotic and abiotic stress tolerance in pearl millet. *Crop Science.* 2021;61(1):289-304.<https://doi.org/10.1002/csc2.20408>
82. Sharma PC, Sehgal D, Singh D, Singh G, Yadav RS. A major terminal drought tolerance QTL of pearl millet is also associated with reduced salt uptake and enhanced growth under salt stress. *Molecular Breeding.* 2011;27(2):207-22.<https://doi.org/10.1007/s11032-010-9423-3>
83. Pongtongkam P, Peyachoknagul S, Manawiboon D, Arananant J, Thongpan A, Tudsri S. Production of salt tolerant Ruzi grass (*Brachiaria Ruziziensis*) by tissue culture. *Agriculture and Natural Resources.* 2006;40(2):449-55
84. Nadir M, Syahrir R, Syamsia, editors. *In Vitro Selection of a Drought Tolerant Callus of Dwarf Napier Grass (Pennisetum purpureum Cv. Mott).* IOP Conference Series: Earth and Environmental Science; 2018: IOP Publishing.
85. Song J-L, Wang Z-Y, Wang Y-H, Du J, Wang C-Y, Zhang X-Q, et al. Overexpression of *Pennisetum purpureum* CCoAOMT contributes to lignin deposition and drought tolerance by promoting the accumulation of flavonoids in transgenic tobacco. *Frontiers in plant science.* 2022;13:884456.<https://doi.org/10.3389/fpls.2022.884456>
86. Meena RP, Ghosh G, Vishwakarma H, Padaria JC. Expression of a *Pennisetum glaucum* gene DREB2A confers enhanced heat, drought and salinity tolerance in transgenic *Arabidopsis*. *Molecular Biology Reports.* 2022;49(8):7347-58.<https://doi.org/10.1007/s11033-022-07527-6>
87. Matthews C, Arshad M, Hannoufa A. Alfalfa response to heat stress is modulated by microRNA156. *Physiologia Plantarum.* 2019;165(4):830-42.<https://doi.org/10.1111/ppl.12787>