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Productivity, forage quality and presence of dehydrin genes in some introduced pasture grass cultivars growing under heat stress in central region of Saudi Arabia

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Abstract

Pasture grass cultivars grown for forage production in the central region of Saudi Arabia, which is dominated with desert environment, suffer a great deal of heat stress. These growing conditions greatly affect species performance and forage quality. This study was focused on evaluating productivity and forage quality of selected new pasture grass cultivars under heat stress in the central region of Saudi Arabia over a two-year period and investigating the presence of the dehydrin genes as a marker of heat tolerance in grass cultivars. Six grass cultivars were used in this study: two perennial ryegrass (*Lolium perenne* L.) cultivars (Aries and Quartet), two endophyte-free tall fescue (*Festuca arundinacea* Schreb.) cultivars (Fawn and K5666v), and two orchardgrass (*Dactylis glomerata* L.) cultivars (Tekapo and Niva). The results clearly showed variation in the total dry matter yield (DMY) and forage quality among the four grass cultivars. Although Fawn tall fescue showed superiority over all other grass cultivars in surviving high temperatures and producing the highest dry matter yield, the forage quality of this cultivar was the lowest among the cultivars examined. SSR markers for the amplification of dehydrin genes revealed the presence of two dehydrin genes (HVDHN1 and HVDHN9) in grass cultivars Aries, Fawn-tall fescue and Tekapo- orchard grass. Fawn had the homologues dehydrin genes. SSR markers for amplifying dehydrin gene could be valuable in grass breeding programs for selection of desirable DHN alleles under heat stress.

Keywords : Heat stress, pasture grass, yield performance, forage quality, dehydrin genes.

Abbreviations: DMY=Dry matter yield, DIP =Diammonium Phosphate, LSD=Least significant differences, CV=Coefficient of Variation, DHNs= Dehydrins

Introduction

The central region of Saudi Arabia is dominantly a desert environment. Climate in this region is hot with average maximum temperatures during summer ranging between 41 to 48^{o}C, and annual rainfall between 100 to150 mm. Agriculture systems are mostly dependent on irrigation using water extracted from aquifers. Alfalfa (*Medicago sativa* L.) is considered the major forage specie in Saudi Arabia. It gives an excellent production of total dry matter yield (DMY) among forage species, which was estimated of two million tons in 2008 (Agriculture statistical year book, 2009). However, due to the extent of livestock production across the country and the higher water use of alfalfa, it is desirable to introduce alternative species that are high in total DMY and forage nutritive value, and low in water consumption. Perennial ryegrass, orchardgrass and tall fescue are cool season plants (C3 plants), which have an optimum growing temperature of 18-24 ^{o}C (Rohweder and Albrecht, 1995). Perennial ryegrass is a temperate species grown throughout the world including North and South America, Europe, New Zealand and Australia. Orchard grass (Cocksfoot in Europe and Australia) is commonly recommended for pastures in the Northeastern and North Central United States. Throughout the world, orchard grass is found in areas of moderate to high rainfall, moderate winter and warm summer (Christie and McElroy, 1995). In addition, it can be grow in low rainfall environment such as the Mediterranean and North Africa region. Tall fescue is used widely for green forage, turf, and conservation purposes (Collins and Hannaway, 2003). It has high yield stability and broad adaptation to different environments, whether grown as monoculture or binary mixtures (Al-Ghumaiz, 2006). The importance of the mentioned grass species is their diversity and adaptation to a wide range of environmental conditions. In addition, cultivars within species have different adaption based on their country of origin. Previous studies indicated that producers should select cultivars that originate from a location with similar climatic conditions (Warnock et al., 2005). Since the indicated species have adaptation to wide range of environmental conditions, producers in Saudi Arabia could benefit from growing such species in their agricultural systems. However, there is no research available to make a valid recommendation on how these species perform under excessive high temperature in the central region of Saudi Arabia. On the other hand, molecular mechanisms involved in stress tolerance of forage and turf grasses are unknown, and studies in this area lag far behind other major crop species. The establishment of synteny among grass genomes based on colinearity of genetic markers, aids the molecular analysis and manipulation of forage grasses (Feuillet and Keller, 2002). Some evidence suggested that genes responsible for a biotic stress resistance in rice (*Oryza sativa* L.) and other cereals have a similar role in forage and turf species (Jiang and Huang 2002; Humphreys et al., 2004). The dehydrin family of proteins accumulates in a wide range of plant species under dehydration stress [late embryogenesis abundant (LEA) D11 family], which range in size from 9 to 200 kDa (Close, 1996). Dehydrins are hydrophilic and heat stable, and may protect other proteins and help maintain the physiological integrity of cells (Bray, 1993). Arora et al. (1998) reported that the accumulation of dehydrin proteins (25-60 kDa) in zonal geranium (*Pelargonium hortorum*, cv. Evening glow) leaves induced by water stress was associated with increased heat tolerance. Turner et al. (2008) described how several QTLs for stress tolerance in *Festuca* were co-located with loci of dehydrin genes (responsible for protecting cells and cell membranes from desiccation). In addition, simple sequence repeats (SSR) marker for amplifying dehydrin gene in conjunction with sequence-characterization RAPD (SCAR) marker for detection of *Fusarium* infection could be a valuable and versatile tool for diagnosis and screening of wheat (*Triticum aestivum*) breeding material for resistance to *F. graminearum* under stress environment (Ibrahim and Motawei, 2008). Our study seeks to evaluate yield performance and forage quality of selected pasture grass cultivars in the central region of Saudi Arabia. Also, the presence of the dehydrin gene as a marker of heat tolerance in grass cultivars was investigated.

Results and discussion

Weather patterns

The average maximum air temperatures at the experiment site during 2008 and 2009 summer months (June, July, August and September) were above long-term average. In 2008, it varied between 42 to 45ºC and ranged from 43.5 to 45.5ºC during 2009. The extremist temperatures recorded during the month of July were 48ºC and 47ºC for 2008 and 2009, respectively. No rainfall was recorded in summer months at both seasons. Relative humidity ranged from 17% to 34% during summer months in both years (Table 2).

Dry matter yield and forage quality

The data showed that there were significant year x cultivars interactions for CP, total DMY and plant height (Table 3). We attribute these interactions primarily to weather conditions varying from year to year and variation among cultivars. No interaction was found for ash, CF and EE analyses. Data analyses showed that there were significant differences ($P<0.05$) in total DMY and plant height among the four cultivars. Fawn-tall fescue had the greatest total DMY (16.92 tons h-1) and highest plant stand (33.34 cm) over all cultivars (Table 4). Significant differences ($p<0.05$) in total DMY were found between the two perennial ryegrasses cultivars (Aries and Quartet) in both years (Table 5). Our study showed that perennial ryegrass cultivars had limited tolerance to heat stress and did not contribute significantly in DMY. These results concur with previous studies conducted in the USA by Leep (2004), who concluded that perennial ryegrass cultivars become dormant during excessive high temperatures even with irrigation or abundant summer rainfall. Similar to perennial ryegrass cultivars, the productivity of Tekapo-orchard grass was low since only three cutting events were taken in both years. Furthermore, comparing the two years performance, our data indicated that there was a trend towards lower total DMY and plant height, that occurred in some cultivars, in 2009 compared to 2008 growing season. As seen from table 5, Aries-perennial ryegrass was 63% lower in total DMY through 2009 vs.2008 (5.75 vs. 9.11 tons h-1) and 66% lower for Fawn-tall fescue (13.5 vs 20.3 tons h-1). In addition, perennial ryegrass cultivars were 50% lower in plant height in 2009 compared to 2008 (12.8 vs. 24.95 cm) (12.77 vs. 26.0 cm) for Aries and Quartet, respectively; 46% lower in Tekapo- orchard grass (14.34 vs. 30.67 cm) and 28% lower for Fawn-tall fescue (12.17 vs. 42.53 cm) (Table 5). Results of forage quality components showed that there were significant differences ($p<0.05$) among cultivars in CP, CF and sugar contents. Although, Fawn-tall fescue survived under high summer temperatures and provided the highest total DMY, yet, it was the lowest in CP and the highest in CF, and that will affect animal intake. This study clearly indicated that some cool season pasture cultivars can be grown in central region of Saudi Arabia. It also indicated that producers should consider these grasses as an annual forage crop, with the exception of Fawn- tall fescue.

SSR marker for dehydrin genes

Dehydrins (DHNs) are one of the typical families of proteins that occur in plants as a consequence of dehydration and osmotic stress (Sivamani et al., 2000). The dehydrin genes were amplified from grass cultivars (Aries, Fawn-tall fescue and Tekapo- orchard grass) using *HVDHN1* and *HVDHN9* primers (Fig. 1). It was shown that Fawn had only one band for *HVDHN9* primers. Therefore, Fawn was homozygous for dehydrin-9 gene. Provan et al. (1999) suggested that co-dominant SSR polymorphism can be detected without the time consuming and laborious processes usually associated with SSR development and characterisation. In addition, the dehydrin gene was amplified from cultivars Aries, Fawn, and Tekapo using *HVDHN*1 primers. On the other hand, SSR analysis using *HVDHN*1 primers did not detect dehydrin gene in Quartet (Fig. 1). However, Fawn-tall fescue had the homologues dehydrin genes. SSR markers for amplifying dehydrin gene could be valuable in grass breeding programs for selection of desirable *DHN* alleles. The enhanced accumulation of dehydrin homologue proteins in the Bermuda (*Cynodon dactylon*) cultivars may function to mitigate heat stress (Zhou and Abaraha, 2007). The same response mechanism also exists in the perennial ryegrass and bentgrass (*Agrostis stolonifera* L.) (Zhou and Abaraha, 2007). The accumulation of the dehydrin proteins might help to protect intact cellular structures under heat stress so that the leaves can maintain a relatively stable level in the chlorophyll and protein contents (Zhou and Abaraha, 2007). Brini et al. (2007) concluded that the observed differential phosphorylation pattern of *HVDHN-5* in the resistant and sensitive wheat varieties could be used as a basis for a molecular screen of tolerance sensitivity to environmental stresses in wheat germplasm. In addition, transgenic rice plants over expressing the barley *(Hordeum vulgare)* in dehydrin gene *HVA*1, showed enhanced tolerance to water and salt stresses (Xu et al., 1996). The co-locations of QTLs controlling water-status and/or turgor with sequences corresponding to dehydrin (*DHN*) genes on the same portion of chromosome 6H, was a great indication of the possible role of these genes in the variation of plant water-status (Teulat et al., 2002). The dehydrins are water-soluble lipid-associated proteins that accumulate in response to dehydration, high temperature, osmotic stress, or during seed maturation (Close, 1996). Several QTLs controlling tolerance traits have already been identified close to dehydrin genes (Campbell and Close, 1997). The first example was for a QTL for winter-hardiness overlapping with a cluster of *DHN* genes, including *HVDHN1* on barley chromosome 5H associated with a cold- specific induction of a member of this dehydrin family (Pan et al., 1994; Zee et al., 1995). Borovkova et al. (1998) showed that a linkage was detected

Table1. Description of the six grass cultivars used in this study

English name	Scientific name	Cultivar name	Country of origin
Perennial ryegrass	*Lolium perenne*	Aries	New Zealand
Perennial ryegrass	*Lolium perenne*	Quartet	New Zealand
Orchardgrasses	*Dactylis glomerata*	Tekapo	New Zealand
Orchardgrasses	*Dactylis glomerata*	Niva	Czech Republic
Tall fescues	*Festuca arundinacea*	K5666v	New Zealand
Tall fescues	*Festuca arundinacea*	Fawn	Oregon -USA

Source: AMPAC seed company-USA.

Table 2. Average daily minimum and maximum air temperatures (^{o}C) for 2008 and 2009 seasons compared with long-term average (27-Years) and mean relative humidity (RH) (%) at the experimental sites

	27-Year Ave.		2008			2009			RH (%)	
Month	T_{min} (°C)	T_{max} (°C)	T_{min} (°C)	T_{max} (°C)	Range (°C)	T_{min} (°C)	T_{max} (°C)	Range (°C)	2008	2009
May	22.4	38.1	24.0	39.5	18 -44	23.7	39.2	18-44	24.5	34.0
June	24.5	41.6	24.2	41.9	20 -45	27.4	43.5	24 -46	18.7	19.6
July	25.5	42.8	26.4	44.2	16 -48	25.2	43.5	23 -47	17.6	22.8
Aug.	25.6	42.7	27.2	44.9	23 -47	26.7	45.6	23 -47	18.1	20.4
Sep.	23.0	40.4	25.0	42.5	20 -46	24.1	42.6	21 -47	22.5	23.3

Source: Presidency of Meteorology & Environmental Protection-Saudi Arabia. http://www.pme.gov.sa

Table 3. Combined analysis of variance for forage quality components (%), total DMY (tons h^{-1}) and plant height (cm) of four grass cultivars in the central region of Saudi Arabia over a two- year period (2008 and 2009 seasons)

SOV	DF	ASH	CF	EE	CP	Sugar	Total DMY	Plant Height
Year (y)	1	0.66	75.54**	0.12	6.85	9.17	42.93**	586.77**
Error	4	1.37	2.62	0.63	3.51	25.15	0.73	7.96
Cultivars (C)	3	1.71	8.35**	1.21**	2.49**	24.65**	148.09**	170.65**
YxC	3	0.01	0.53	0.13	1.94**	2.27	14.73**	48.95**
Error	12	0.62	0.36	0.06	0.25	0.81	2.38	8.27
CV %		6.16	2.35	5.51	3.97	2.05	16.26	11.02

** $P<0.01$. DF= Degree of Freedom,CF= Crude Fiber, EE= Ether Extract, CP = Crude Protein ,DMY=Dry Matter Yield.

between leaf rust resistance gene *Rph9* and the microsatellite marker dehydrin-9 (*HVDHN9*) at a distance of 10.2 cM in the Bowman/Hor 2596 cross.

Materials and methods

Site description and trial establishment

Field experiments were conducted under irrigation conditions during 2008 and 2009 seasons at Qassim University Agricultural Research and Experimental Station (26° 18 28 N, 43° 46 E). The site of the experiment was sandy loam soil that contained low level of soluble salts (EC=1.5 dsm^{-1}) and low organic matter (1.3%) with pH value of 8.1. Cool season grass cultivars were established in a randomized complete block design (RCBD) with three replications using 3 m^{2} plot (ten rows per plot) with seeding rate of 20kg ha^{-1}. Each replication consisted of six grass cultivars as follows: two perennial ryegrass, two orchardgrass, and two endophyte-free tall fescue cultivars. Two cultivars (Niva-orchard grass and K5666v-tall fescue) were excluded from data analyses because of their poor establishment in both years. Table 1 listed the name and country of origin of species and cultivars used in this study. Planting dates were in January 5, 2008 and December 27, 2009 for the first and second season, respectively. Soil was analyzed at Qassim University Soil Testing Laboratory and fertilizers were applied as recommended by soil test results. Nitrogen (N) was applied to all plots following each cutting in the form of Diammonium Phosphate (DIP) at a rate of 50.4 kg ha^{-1}. Average daily maximum and minimum temperatures and relative humidity for 2008 and 2009 growing seasons are presented in Table 2.

Data collection and sampling procedure

Forage biomass samples were hand clipped within a 0.30 m^{2} in each plot. Three to four cutting events were obtained with 30 to 50 days interval between each one. The cutting schedule of 2008 season was in 12-April, 24-May and 14-July, 2008, with additional cuts taken from Fawn-tall fescue cultivar in 31-August and 30-October, 2008. In 2009, cutting schedule took place in 23-April, 28-May, 25-June, and 1st -August 2009 and the extra cuts from Fawn were taken in September 5 and October 17. The clipped samples were oven-dried at 60ºC for 72 hr, then weighed for DMY (tons h^{-1}). Dried samples were analyzed for the following parameters: Crude protein (CP %), crude fiber (CF %), sugar content (%), ether extract (EE) (%), and ash (%). Prior to each sampling procedure, average plant heights (cm) were determined by measuring the most representative area of each plot. Chemical analyses were completed at Forage Analyses Laboratory- Department of Animal Production and Breeding-Qassim University, according to procedures used by AOAC (1990). Foss TECATOR apparatus (Model:2300 Kjeltec) was used to measure CP and Model :Fibertec2010 for CF analysis.

DNA extraction

Bulk leaf samples of each grass cultivar were collected and ground into fine powder with liquid nitrogen. The DNA

Table 4. Forage quality components (%), total DMY(tons h^{-1}), and plant height (cm) of the four new grass cultivars growing in the central region of Saudi Arabia over a two-year period (2008 and 2009 seasons)

Species	cultivar	ASH	CF	EE	CP	Sugar	Total DMY	P.Height
P.ryegrass	Aries	12.45	24.71c	4.64a	11.98b	45.43a	7.43b	21.36c
	Quartet	13.59	24.61c	4.42a	12.09b	44.25a	6.79b	22.92c
Orchard.G	Tekapo	12.82	27.16a	4.72a	13.40a	41.00b	6.78b	26.75b
Tall fescue	Fawn	12.45	25.53b	3.73b	12.48a	45.10a	16.92a	33.34a
	LSD	NS	0.75	0.30	0.62	1.14	1.94	3.62

Mean values within each column followed by the same letter(s) are not statistically different at *P<0.05* level according to Duncan's Multiple Range Test.

Table 5. Effect of interaction between years and cultivars in CP (%), total DMY(tons h^{-1}) and plant height (cm) of the four new grass cultivars growing in the central region of Saudi Arabia over a two years period (2008 and 2009 season)

Species	cultivar	CP		Total DMY		Plant Height	
		2008	2009	2008	2009	2008	2009
P.ryegrass	Aries	11.16b	12.80b	9.11c	5.75d	24.95cd	12.80b
	Quartet	11.42cd	12.77b	7.03cd	6.55cd	26bc	12.77b
Orchard G	Tekapo	12.46b	14.34a	6.79cd	6.76cd	30.67bc	14.34a
Tall fescue	Fawn	12.78b	12.17bc	20.33a	13.50b	42.53a	12.17bc
	LSD	0.88		2.74		5.11	

Mean values within each trait followed by the same letter(s) are not statistically different at *P<0.05* level according to Duncan's Multiple Range Test. CP = Crude Protein

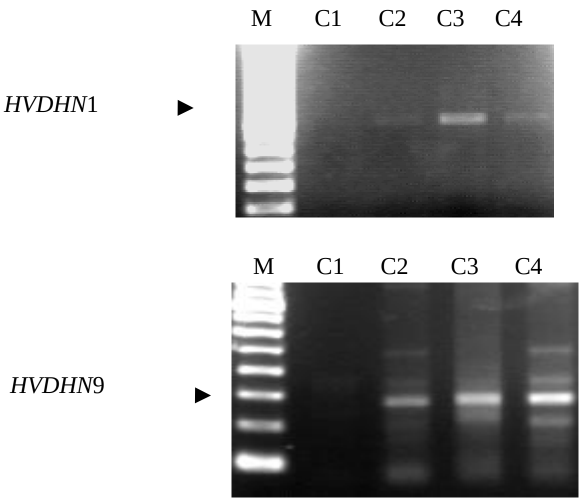

Fig 1. Detection of dehydrin genes in grass cultivars (C1= Quartet, C2= Aries, C3= Fawn, C4= Tekapo) using SSR primer (*HVDHN*1 and *HVDHN*9). M is a ladder marker (100bp). Specific primers for gene *HVDHN*1 amplified with grass cultivars Aries, Fawn, and Tekapo. Arrow shows the one polymorphic band for *HVDHN*1gene. The PCR products of the *HVDHN*9 gene were present in cultivars Aries, Fawn, and Tekapo. Fawn produced one band for *HVDHN*9 gene.

extraction was done using CTAB method (Sagahi-Maroof et al. 1984; Al-Doss et al. 2010). The DNA concentration was assessed spectrophotometrically at 260 nm, and quality was assessed by the 260/280 ratio (Sambrook et al. 1989). The DNA was suspended to a final concentration of 10 ng/µl in 0.5X TE and stored at 4ºC.

Simple sequence repeats (SSR) marker for dehydrin gene

SSR primers for amplification of dehydrin genes were *HVDHN1* and *HVDHN9* primers. These primers were designed by Pharmacia Biotech, Germany, on the basis of the published sequence (Becker and Heun, 1995). Amplification was carried out in 25 µL reaction volumes, containing 1X Taq polymerase buffer (50 mM KCl, 10 mM Tris, pH 7.5, 1.5 mM MgCl2) and 1 unit of Taq polymerase (Pharmacia Biotech, Germany) supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 25 pmol of forward and reverse of each primer, and 50 ng of total genomic DNA. Amplification was performed in a thermal cycler (Thermolyne Amplitron) programmed for 1 cycle of 30 secs at 94˚C; 40 cycles of 1 min at 94˚C, 1 min at 55˚C and 1 min at 72˚C; followed by 5 min at 72˚C.

Data analysis

Analysis of variance (ANOVA) was performed, and the four cultivars means were compared using the least significant differences (LSD) at *P*<0.05. Coefficient of variation (CV) was listed to measure the precision of the trial. All statistical computations were performed using the MSTATC microcomputer program (MSTATC, 1990).

Conclusion

This study demonstrated a variation between grass cultivars in their adaption under high summer temperatures. Fawn-tall fescue exhibited excellent yield stability during the two years course of study and appeared to be well adapted to the central region of Saudi Arabia. However, it had low forage quality compared to other cultivars. Niva-orchard grass and K5666v-tall fescue had a poor establishment under the conditions of the central region of Saudi Arabia, while others such as Aries and Quartet-perennial rye grass cultivars demonstrated a limited tolerance when grow under similar environment condition. In addition, Fawn-tall fescue had the homologues dehydrin genes and produced the highest total DMY. Therefore, SSR markers for amplifying dehydrin gene could be valuable for grass breeding programs for selection of desirable DHN alleles under heat stress.

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