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Original Article

Water productivity and growth parameters of Fawn-tall fescue and Tekapo-orchard grass under deficit irrigation in arid zones

Produtividade da água e parâmetros de crescimento de *Festuca Fulvo-Tall* e *Capim-pomar Tekapo* sob irrigação com déficit em zonas áridas

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Abstract

This study aimed to determine the drought stress response of Fawn-tall fescue and Tekapo-orchard grass and investigate a drought stress resistance marker. Grass genotypes were grown under four Irrigation treatments *I1* equivalent to 0.3 standard crop evapotranspiration (*ETc*), *I2* equivalent to 0.65 *ETc*, *I3* equivalent to 0.75 *ETc*, and *I4* equivalent to 1.2 *ETc*. Plant height, fresh weight, dry weight were measured and the Water productivity (*WP*) were calculated. The results showed a reduction in the growth of both grass genotypes as the drought stress increased as indicated by the shorter plants and reduction in fresh and dry weight. However, the *WP* results showed that the Fawn-tall fescue endured the drought stress better than the Tekapo-orchard grass as indicated by the constant values of the plant *WP* across the tested irrigation treatments. The results was confirmed by the amplification of dehydrin genes where Fawn-tall fescue was found to be homozygous for dehydrin genes.

Keywords: Fawn plant, Tekapo plant, water deficit, dehydrin, genetic variability, drought stress.

Resumo

Este estudo teve como objetivo determinar a resposta ao estresse hídrico da *Festuca Fawn-tall* e do Capim-pomar *Tekapo* e investigar um marcador de resistência ao estresse hídrico. Genótipos de gramíneas foram cultivados sob quatro tratamentos de irrigação em que I1 é equivalente a 0,3 da evapotranspiração padrão da cultura (ETc), I2 equivalente a 0,65 ETc, I3 equivalente a 0,75 ETc, e I4 equivalente a 1,2 ETc. Altura da planta, peso fresco, peso seco foram medidos e a produtividade de água (WP) foi calculada. Os resultados mostraram uma redução no crescimento de ambos os genótipos de gramíneas à medida que o estresse hídrico aumentou, conforme indicado pelas plantas mais baixas e redução no peso fresco e seco. No entanto, os resultados do WP mostraram que a espécie *Festuca Fulvo-Tall* suportou o estresse hídrico melhor do que a grama Capim-pomar *Tekapo*, conforme indicado pelos valores constantes do WP da planta em todos os tratamentos de irrigação testados. Os resultados foram confirmados pela amplificação dos genes da deidrina, em que a Festuca *Fulvo-Tall* foi encontrada e classificada como homozigótica para tais genes.

Palavras-chave: *Fulvo-tall*, *Tekapo*, déficit hídrico, deidrina, variabilidade genética, estresse hídrico.

1. Introduction

Water deficit is by far the most important environmental factor contributing to yield losses in crops (Weber et al., 2014). Turf grass plants experience drought stress either when the water supply to roots becomes difficult or the transpiration rate becomes very high. These two conditions often coincide under arid and semiarid climates (Weber et al., 2014). Drought stress has become more of a problem for production of turf and forage grasses. The intensity of drought stress may also increase as a result of climate change, which impact growth and persistence of perennial grasses (Cui et al., 2015). Moreover, cool-season perennial grass species normally require large amount of water to maintain growth. Therefore, improvement of drought tolerance of turf and forage grasses is becoming increasingly important to minimize effects of drought on plants.

Tall fescue (*Festuca arundinacea* Schreb) is one of the most important forage grown extensively in Europe and the United States. Tall fescue is considered a perennial crop, where they may remain in the soil from 3 to 6 years

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and grow under summer heat and high humidity as well as have the ability to adapt to different environment conditions. Tall fescue is also known with its' improved drought and heat tolerance it is a cool-season grasses that maintain year-round green color (Miller et al., 2016). Tall fescue has high persistence (Berzins et al., 2018), and is drought tolerant (Cougnon et al., 2014; Cross et al., 2013). Therefore, tall fescue cultivars may be of interest to forage grass breeders due to their high regrowth capacity and nutritive value (Ostrem et al., 2013). Only 3% of the turf grass water intake is dedicated to its physiological process and the rest of the water intake is used as transpiration. The factors affecting turfgrass transpiration rate are the internal leaf diffusion resistance, boundary layer resistance, and vapor pressure gradient between the air and leaf (Fry and Huang, 2004).

Turfgrass water use is the combined amount of plant transpiration and soil evaporation, and because of the nature of turfgrass plantations the transpiration is the dominate component in the evapotranspiration but studies have shown that considerable amount of water can be lost in evaporation during darkness (Rosas-Anderson et al., 2018). The evapotranspiration rates of the tall fescue reached 9.7 mm d^{-1} in arid and greenhouse environments (Bowman and Macaulay, 1991) and reached a maximum value of 6.1 mm d-1 in less arid conditions (Carrow, 1996). Although several water use management techniques such as soil moisture sensors the main method of effective plant water use determination remains the use of empirical models and metrological data records (Wherley et al., 2015). Exploring the molecular mechanisms of drought stress in turf grass can contribute to improving their drought tolerance in application research. Many candidate genes related to drought tolerance were identified in turf grass (Fan et al., 2020). Xu et al. (2017) reported that the overexpressing an Arabidopsis *DREB1A* in Kentucky bluegrass showed higher drought tolerance than control. In addition, Al-Ghumaiz and Motawei (2011) concluded that the dehydrin gene could be valuable in grass breeding program for selection of desirable DHN alleles under heat stress.

Fawn- Tall fescue is one of the cool season cultivar that had a promising results when evaluated in Qassim region (Al-Ghumaiz and Motawei, 2011; Motawei and Al-Ghumaiz, 2012). A second study, investigated the effect of different levels of irrigation water salinity on a number of grass cultivars that was able to show coping in Qassim region environment. Fawn showed an ability to continue growing up to 8000 ppm (Al-Ghumaiz, 2013; Al-Ghumaiz et al., 2017), but the response of Fawn to deficit irrigation under the arid conditions of Qassim region needed to be investigated and determined. Tekapo-orchard grass is one of the important perennial forage grasses worldwide the plant can adapt to different local, it has shade tolerance, and moderate drought resistance (Abtahi et al., 2017). Olson et al. (2007) said that orchard grass (*Dactylis glomerata* L.) is a cool-season, perennial, tall growing, grass that is shade-tolerant, but will grow well in full sunlight. Saeidnia et al. (2018) discussed the effect of drought stress on orchard grass they reported a correlation between the drought stress and the reduction of dry mass yield, relative water content, and chlorophyll content. The previous studies suggest that Fawn- Tall fescue is a forge crop that can endure drought (Cougnon et al., 2014; Cross et al., 2013) and would be a suitable forge crop to implement in arid zones where water supply might be a problem during some periods of the year. This research aims to study the effect of deficit irrigation on yield and water productivity of Fawn-Tall fescue plants and compare it with another high value cool-season perennial (Tekapo-orchard grass), then assure the results by investigating the presence of the dehydrin gene as a marker of drought tolerance in grass cultivars.

2. Material and Methods

2.1. Plant materials and the experimental design

The field experiment was conducted at Qassim University Agricultural Research and Experimental Station (26°17'51.5"N 43°45'54.9"E). The experiment included planting Fawn-Tall fescue (*Festuca arundinacea*) and Tekapo-orchard grass (*Dactylis glomerata*). The experimental design (Figure 1) was a split plot design Randomized Complete Block Design (RCBD) with three replications, 1 m^2 plot (ten rows per plot) each with seeding rate of 20 kg ha^{-1}. The whole plot factor was the varieties under study, and the subplot factor was the irrigation levels. A blank plot (1 m in each direction) was used to separate the experimental units; one hp hydraulic pump was used to deliver water to the experimental units. The pump delivered the water to a 50 mm polyethylene main line with four sub main lines 32 mm in diameter. The beginning of each sub main (water treatments) was equipped with a water flow-meter to measure the amount of water entering each treatment and a stopcock to control the irrigation amount.

2.2. Irrigation treatments for the experiment were as follows

1. *I1* equivalent to 0.3 standard crop water requirements in the study area "*ETc*"

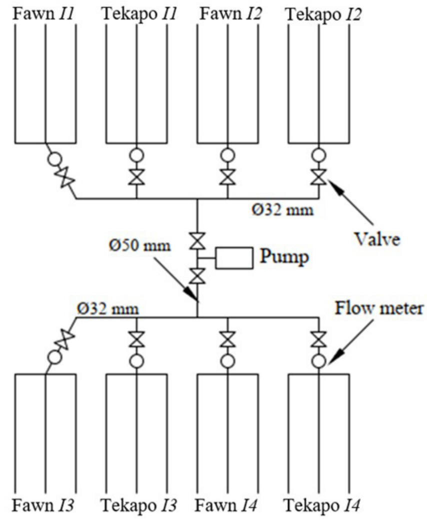

Figure 1. Experimental layout.

2. *I2* equivalent to 0.65 standard crop water requirements in the study area "*ETc*"
3. *I3* equivalent to 0.75 standard crop water requirements in the study area "*ETc*"
4. *I4* equivalent to 1.2 standard crop water requirements in the study area "*ETc*"

Crop evapotranspiration was calculated using the FAO / Penman - Monteith equation using the computer program "CropWat" developer by the Smith et al. (2002) and the available metrological data records for the study area (Historical metrological data records from 1978 to 2013).

The effective rain during the growing season were calculated using the Equations 1 and 2 according to SCS (1971).

$$ER = SF \times \left[\left(\left(0.70917 \times \left(\frac{Pm}{25.4}\right)^{0.82416} - 0.11556\right) \times 10^{0.000955 ET_c}\right) \times 25.4\right] \quad (1)$$

where:
ER = Monthly estimation of effective rain, mm;
SF = soil water storage factor;
Pm = average monthly precipitation, mm;
ET_C = average monthly crop evapotranspiration, mm;
SF = was calculated using equation.

$$SF = 0.531747 + 0.295164\left(\frac{D}{25.4}\right) - 0.057697\left(\frac{D}{25.4}\right)^2 - 0.003804\left(\frac{D}{25.4}\right)^3 \quad (2)$$

D = usable soil water storage
$D = 0.66\,AWHC$
AWHC = is the available water holding capacity $cm^3\ cm^{-3}$

The effective rain *ER* was deducted from the amount of water added to the treatment during the experiments.

2.3. Measurements

1- Plant height (cm) 2-Fresh weight (Kg ha^{-1})
3- Dry Matter Yield (Kg ha^{-1}).
4- Total irrigation water ($m^3\ ha^{-1}$)
5- Water productivity (*WP*) were calculated according to Geerts and Raes (2009).

2.4. Data analysis

Analysis of variance (***ANOVA***) was performed, and the grass genotypes and the irrigation means were compared using the least significant differences (***LSD***) at $P<0.05$. All statistical computations were performed using (SAS Institute, 2013).

2.5. DNA extraction

Bulk leaf samples of each grass cultivar were collected and ground into fine powder with liquid nitrogen. The *DNA* extraction was done using CTAB method (Saghai-Maroof et al., 1994). The *DNA* concentration was assessed spectrophotometrically at 260 nm, and quality was assessed by the 260/280 ratio (Sambrook et al., 1989).

2.6. 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.

3. Results and Discussion

3.1. Effect of grass genotypes and irrigation levels on plant height, yield and water productivity

The seasonal amount of irrigated water supplied to each irrigation treatment for both Fawn and Tekapo-orchard grass are shown in Table 1. It's clear that the amount of water supplied to the plants decreased from treatment *I4* to *I1* putting a drought stress on the plants.

The Tekapo-orchard grass had lower average plant height than Fawn for all irrigation treatments in the experiment (Table 2). The overall plant height averages of all irrigation treatments were 15.42 cm for Fawn plants and 11.85 cm for Tekapo-orchard grass. However, the results showed that the effect of genotypes was insignificant (Table 2). From Table 2 it's also clear that the irrigation level had a significant effect on the growth and the yield of both types of grass. *I4* treatment had a significantly higher average plant height than irrigation treatments *I2* and *I1* but it was not significantly higher than treatment *I3*. The same trends were found for the fresh matter yield and the dry matter yield. The average fresh matter yield and dry matter yield of both grasses genotypes were insignificant from each other, while the irrigation treatment *I4* had a significantly higher average fresh and dry matter yield than all the other irrigation treatments. The *I3* irrigation treatment had significantly higher average fresh weight than *I2*, and *I1* irrigation treatments. *I2* and *I1* irrigation treatments had a fresh matter yields that were insignificant from each other. For the dry weight yield, *I4* was significantly higher than

Table 1. The seasonal amount of irrigation water for both Fawn and Tekapo-orchard grass.

Treatment	Seasonal amount of irrigation water ($m^3\ ha^{-1}$)	
	Fawn	Tekapo-orchard grass
I1	2298.128	2673.2
I2	3457.133	5721.3
I3	6307.673	5694.6
I4	10074.82	7605.1

Table 2. The effect of irrigation treatments and genotypes on plant height, fresh and dry matter yield and the water productivity of the two grasses.

Treatment	Plant height (cm)	Fresh matter yield (kg ha^{-1})	Dry matter yield (kg ha^{-1})	*WP* (kg.m^{-3})
Genotypes (G)				
Fawn	15.42^{a}	1467.5 ^{a}	512.9 ^{a}	0.092 ^{a}
Tekapo	11.85^{a}	1324.0 ^{a}	468.3 ^{a}	0.087 ^{a}
Sig	n.s.	n.s.	n.s.	n.s.
Irrigation treatments (*I*)				
I1	11.83 ^{b}	465 ^{c}	183.3 ^{c}	0.0761 ^{ab}
I2	12.67 ^{b}	823.2 ^{c}	362.2 ^{bc}	0.0629 ^{b}
I3	15.33 ^{ab}	1736.7 ^{b}	541.8 ^{b}	0.114 ^{a}
I4	17.50 ^{a}	2558.3 ^{a}	875.2 ^{a}	0.107 ^{ab}
Sig	**	***	***	*
I×G	**	*	***	**

n.s. Not significant at the 0.05 and 0.01 probability levels. * Significant at the 0.05 probability levels. ** Significant at the 0.01 probability levels. *** Significant at the 0.001 probability levels. $^{a, b, c}$ Means within the same column for each factor followed by similar letters are not significantly different at $P < 0.05$.

all the other irrigation treatments. *I3* and *I2* treatments had dry weight matter insignificant of each other but *I3* was significantly higher than *I1*. The genotypes had no significant effect on the *WP*. Fawn had a higher average *WP* than the Tekapo but the differences in the *WP* were insignificant. The *I2* treatment had the highest *WP* among all the treatments. The value of *WP* of the *I3* irrigation treatment was significantly higher than the irrigation treatments *I2*, but it was insignificant from the *WP* value of the other irrigation treatments.

3.2. The effect of interaction between grass genotypes and irrigation levels on plant height, yield and water productivity

The interaction between the genotypes and the irrigation treatments had a significant effect on all the measured plant properties in this experiment (Table 2). The effect of irrigation treatments on the water productivity of grasses genotypes were shown in Figure 2. It's clear that the *WP* trends of the two grasses "Fawn and the Tekapo" were different. *WP* of the Tekapo decreased as the level of irrigation decreased from *I4* to *I2*. For the genotype "Fawn", the value of the *WP* remained almost the same for all the irrigation treatments and the differences in the *WP* between all the irrigation treatments were insignificant (Figure 2).

The fresh weight of Fawn decreased significantly when the level of irrigation decreased going from treatment *I4* to *I3*. However, the fresh weight of genotype "Fawn" decreased insignificantly from treatment *I2* to *I1*. The reductions in the fresh weight calculated as percentage from the fresh weight for irrigation treatment *I4* were 32, 41, 71% for irrigation treatments *I3*, *I2*, and *I1* consequently. For genotype "Tekapo", the fresh weight decreased significantly as the level of irrigation decreased from treatment *I4* to *I2* then it was insignificantly decreased for *I1* (Figure 3). The reductions in the fresh weight calculated as percentage from the fresh weight for irrigation treatment *I4* were 32, 86, 88% for irrigation treatments *I3*, *I2*, and *I1* consequently. The dry weight of the genotype "Fawn" decreased also when the irrigation decreased going from treatment *I4* to *I1*. The reductions in the dry weight calculated as percentage

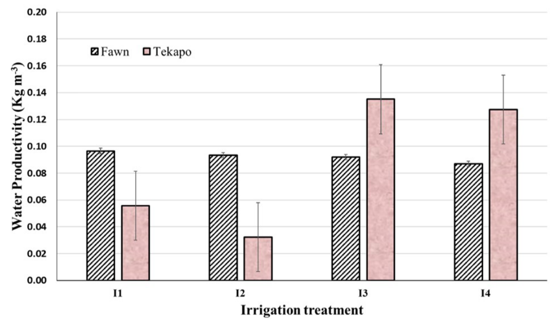

Figure 2. Water Productivity of Fawn-Tall fescue and Tekapo-orchard grasses under irrigation treatments.

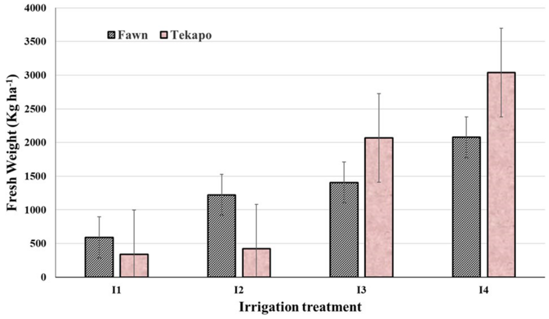

Figure 3. Fresh weight of Fawn-Tall fescue and Tekapo-orchard grasses under irrigation treatments.

from the dry weight for irrigation treatment *I4* were 26, 58, 71% for irrigation treatments *I3*, *I2*, and *I1* consequently. The dry weight of the Tekapo significantly decreased as the irrigation decreased (Figure 4). The reductions in the dry weight calculated as percentage from the dry weight for irrigation treatment *I4* were 23, 80, 84% for irrigation treatments *I3*, *I2*, and *I1* consequently.

The plant height had a similar trend as the dry weight. The plant height of the Fawn decreased when the irrigation decreased going from treatment *I4* to *I1*. The reductions in the plant height calculated as percentage from the plant height for irrigation treatment *I4* were 21, 33, 36% for irrigation treatments *I3*, *I2*, and *I1* consequently. The plant height of the Tekapo decreased when irrigation decreased (Figure 5). The reductions in the dry weight calculated as percentage from the dry weight for irrigation treatment *I4* were 7.5, 36, 44% for irrigation treatments *I3*, *I2*, and *I1* consequently.

3.3. Detection of dehydrin genes in grass genotypes

Dehydrin genes were detected in both grass genotypes (Fawn-tall fescue and Tekapo-orchard grass) using *SSR* markers (*HVDHN1* and *HVDHN9*) (Figure 6). It was shown that Fawn-Tall fescue had only one band for both *HVDHN1* and *HVDHN9* markers. However, Tekapo-orchard grass had three bands *HVDHN9* marker. Therefore, Fawn-tall fescue was homozygous for dehydrin genes.

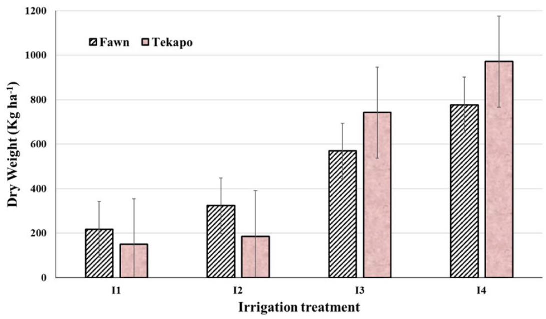

Figure 4. Dry weight of Fawn-Tall fescue and Tekapo-orchard grasses under irrigation treatments.

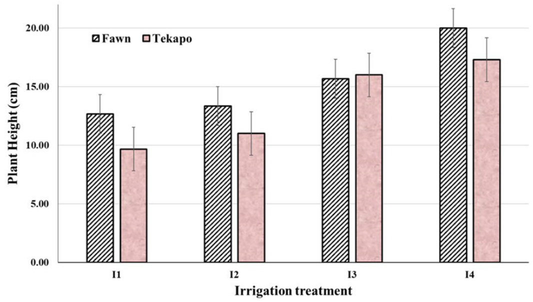

Figure 5. Plant height of Fawn-Tall fescue and Tekapo-orchard grasses under irrigation treatments.

4. Discussion

In light of the results of previous research investigating the fawn response to heat and salinity stresses (Al-Ghumaiz et al., 2017). It was assumed in this research that Fawn-Tall fescue will perform better under drought conditions than the Tekapo-orchard grass and it will achieve a higher *WP* than the Tekapo-orchard grass under deficit irrigation conditions. Tall fescue is considered a perennial crop; it may remain in the soil from three to 6 years and grow under summer heat and high humidity. This makes it a good candidate to be used as a forge or even as turfgrass in hot zones. On the other hand Tekapo-orchard grass is considered a leading forge crop because of its high nutritional value and its yield. The responses of the two kinds of grass genotypes to heat, salinity, water stress under the conditions of arid zones had to be investigated to determine if they will grow successfully in the region (Al-Ghumaiz et al., 2017). In previous investigation, the genotype "Fawn-tall fescue" showed good tolerance to heat and high salinity (Al-Ghumaiz et al., 2017). This work investigated both grass genotypes (Fawn-tall fescue and Tekapo-orchard grass) response to water stress and explored the causes of such response. The results showed a decrease in total fresh weight and the heights of the plants as the irrigation supplied to the plants decreased for both grass genotypes (Fawn-tall fescue and Tekapo-orchard grass); indicating a decreased plant growth as a response to water stress. The percentage of reduction in the fresh weight were for the two genotypes were the greatest among all the measured parameters this is because of the direct effect of the water deficit on the hydration states of the plants. The comparison between the effects of going from one irrigation to the other on the two genotypes shows that from *I4* to *I3* both plants had almost the same percentage of reduction in the fresh weight while from *I3* to *I2* Tekapo-

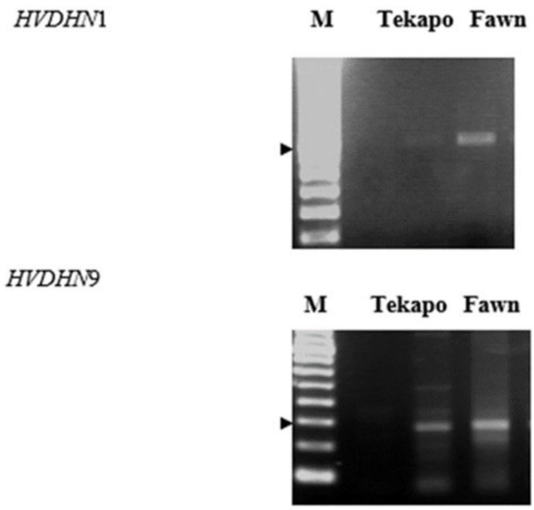

Figure 6. Detection of dehydrin genes in grass genotypes (Fawn and Tekapo) using *SSR* primer (*HVDHN*1 and *HVDHN*9). M is a ladder marker (100bp). Arrow shows the one polymorphic band for *HVDHN*1gene.

orchard grass had a much higher fresh weight reduction than Fawn-tall fescue. Then from *I2* to *I1* the reduction in Fawn-tall fescue was sharper than the Tekapo-orchard grass but the overall of the reduction in the percentage of the fresh weight of the Tekapo-orchard grass remained bigger the overall percentage of reduction in the fresh weight of the Fawn-tall fescue. This suggest that the Fawn-tall fescue has some type of drought tolerance mechanism that was presented more clearly as the irrigation water decreased. The results of the percentage of reduction in the dry weight of grass genotypes (Fawn-tall fescue and Tekapo-orchard grass) also indicate the same response for the two genotype with a sharp percentage of reduction in the dry weight between *I4* and *I3* for Tekapo-orchard grass and the overall of the reduction in the percentage of the dry weight of the Tekapo-orchard grass remaining bigger the overall percentage of reduction in the dry weight of the Fawn-tall fescue. These results are in accordance with the results of Asay et al. (2001) who reported a significant difference in the dry matter yield of tall fescue across four levels of irrigation. The results of the water productivity of both grass genotypes (Fawn-tall fescue and Tekapo-orchard grass) showed a different trend. The genotype "Tekapo" was higher in its water productivity than the genotype Fawn-tall fescue at the irrigation treatments *I4* and *I3* but after that, the water productivity of the genotype "Tekapo-orchard grass" decreased sharply while the water productivity of the genotype "Fawn-tall fescue" remained almost constant for all the irrigation treatments. The results are different from what Cheng et al. (2021) reported, they found that the *WP* of maize increased under deficit irrigation conditions. The dissimilarity in the results may be because of the variation in the nature of the harvested part (grains in maize and the shoot in both Tekapo and Fawn). The results suggest that the genotype "Fawn-tall fescue" had a higher drought tolerance than the genotype "Tekapo-orchard grass". These results concur with previous studies conducted by Schiavon et al. (2014), who studied the effect of drought on cool season turf grass in a sandy soil. There results showed an improvement in Fawn response to drought indicating a plant adaptation to drought stress.

Dehydrins (*DHNs*) are embryonic abundant proteins described by the dehydrin domains that plays an important role in combating dehydration stress (Zhou et al., 2019). The results obtained strongly indicated the importance of *DHNs*, as they are conserved during drought stress. It was noted that Fawn–tall fescue had homozygous dehydrin genes using *HVDHN1* and *HVDHN9* markers. In addition, the *WP* of Fawn–tall fescue increased insignificantly from *I1* to *I2* treatments after those treatments *I2*, *I3*, and *I4* had the same *WP*. However, the *WP* of Tekapo-orchard grass decreased as the level of irrigation decreased from *I1* to *I3*. Al-Ghumaiz and Motawei (2011) concluded that Fawn-tall fescue had the homologues dehydrin genes and produced the highest total dry matter yield under heat stress. In addition, Kamara et al. (2021) found that wheat genotypes "Gammeza11 and Line 109" were the highest homologies for dehydrin gene sequence with heat and drought-tolerant species. Therefore, a positive relationship between the expression levels of *DHNs* transcripts or proteins and plant stress tolerance has been verified and usually more tolerant genotypes or cultivars have higher levels of *DHNs* than the less tolerant ones (Kosová et al., 2012). Furthermore, an enhanced tolerance to osmotic stresses was also observed in Arabidopsis transgenic plants over expressing the dehydrin *DHNs* of *Triticum durum* (Brini et al., 2011). Based on all these results, *DHNs* feature as useful tool to develop stress tolerance genotype.

5. Conclusion

This study demonstrated a variation between both grass genotypes (Fawn-tall fescue and Tekapo-orchard grass) in their adaptation under water stress. Fawn-tall fescue exhibited more dry weight yield than Tekapo-orchard grass under deficit of water irrigation. Also, the water productivity of the genotype "Tekapo-orchard grass" decreased sharply while the water productivity of the genotype "Fawn-tall fescue" remained almost constant for all the irrigation treatments. In addition, Fawn-tall fescue had the homologues dehydrin genes (*HVDHN1* and *HVDHN9*) and produced higher yield than Tekapo-orchard grass under deficit of water irrigation. Therefore, *SSR* marker for amplifying dehydrin genes could useful tool to develop stress tolerance grass genotype.

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