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Title: TEMPERATURE X GENOTYPE EFFECTS FOR PERLOLINE, CRUDE PROTEIN AND TOTAL WATER SOLUBLE CARBOHYDRATE LEVELS IN TALL FESCUE (FESTUCA ARUNDINACEA SCHREB.)

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Abstract approved: Dr. R. V. Frakes

Perloline levels have been associated with poor animal performance on tall fescue pastures. Animal performance is low when perloline levels are high. High perloline levels are reported during hot, dry weather and increased N fertilization. Tall fescue perloline levels appear to be affected by genetic and environmental differences. Positive associations between perloline and protein have been reported and negative associations between protein and water soluble carbohydrate (WSC) levels were also observed. Crude protein and WSC are factors also affected by environmental changes. A study of relationships between these factors will help properly assess forage quality in tall fescue.

Four tall fescue genotypes were placed at random in control climate chambers with 12-hour light periods, at 15°, 20°, and 30°C. Forage harvests were taken at three 28-day

intervals and analysed for perloline, crude protein and WSC. Significant genotype x temperature interactions for all of the measured components were observed.

Averaged over genotypes, there was generally an increase in perloline and crude protein and a decrease in WSC with increasing temperature. Correlation coefficients, within genotypes, among levels of components, indicate that perloline is positively associated with crude protein. This association, however, was statistically significant for only one genotype. Also, only one genotype exhibited a significant association between crude protein and WSC, although crude protein and WSC are negatively associated. Associations between perloline and WSC were also negative but not statistically significant.

The results indicate that genotype selection for quality components is feasible. Genotype screening at different temperatures may be an important procedure in the identification of parent genotypes in a tall fescue quality improvement program. Selection for material with the genetic potential for low of high perloline level can be accomplished at high temperatures, near 30°C, while selection for crude protein and WSC should be done at lower temperatures, from 15° - 20°C.

Temperature x Genotype Effects for Perloline, Crude Protein and Total Water Soluble Carbohydrate Levels in Tall Fescue (Festuca arundinacea, Schreb.)

by

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TEMPERATURE X GENOTYPE EFFECTS FOR PERLOLINE, CRUDE PROTEIN AND TOTAL WATER SOLUBLE CARBOHYDRATE LEVELS IN TALL FESCUE (*FESTUCA ARUNDINACEA*, SCHREB.)

Perloline is the major alkaloid of tall fescue (*Festuca arundinacea*, Schreb.) and is found in high concentrations in perennial ryegrass (*Lolium perenne*, L.) and several other grass species (9, 16). It is believed to be derived from tryptamine (10) and, therefore, contains nitrogen. Perloline is reported to be physiologically active and mildly toxic to ruminants (23), in that it appears to inhibit ruminal digestion and volatile fatty acid production. Bush *et al.* (6) have demonstrated the effects of high perloline concentrations on *in vitro* rumen bacteria. Bush and Buckner (7) found that poor animal performance on tall fescue corresponded to periods of high perloline levels in the forage. Perloline content is usually high during periods of rapid forage growth and Gentry *et al.* (13) report high perloline accumulation in periods of hot, dry weather and with increased nitrogen fertilization.

Gentry *et al.* (13) found differences in perloline content among and within varieties of tall fescue and in *Lolium* and *Lolium* x *Festuca* hybrids. Significant genotype x environment interactions have been reported for perloline content in tall fescue (22) but Reifer and Bathurst (19) reported that no single environmental factor could account

for the variation in perloline content. Perloline is reported to be controlled by relatively few genes with dominance for low concentration (4, 5, 6, 11, 22). Watson (22) reported a significant additive genetic effect for perloline content. High and low perloline level experimental lines have been developed in Lolium x Festuca hybrids and tall fescue (4). However, animals are reported to perform better while grazing the high perloline forage in comparison to forage with lower perloline levels (24).

Bennett (2) found that nitrogen content of perennial ryegrass was positively associated with perloline. Significant differences in nitrogen content among tall fescue cultivars have been reported (17, 22). Percent nitrogen is used as a measure of crude protein content in forages and tall fescue is reported to be relatively low in crude protein (7, 12, 17). Crude protein or percent nitrogen is negatively correlated with percent carbohydrates (4, 14). Reduced carbohydrate levels have been observed with increased temperatures (20). Since soluble carbohydrates account for major differences in digestibility among forage species (12), their low levels may account for poor animal performance during periods of high temperature.

Associations between these quality factors are evident, and they appear to be affected by temperature and related to animal performance. Based on the reports of significant genotype x environment interactions for perloline content

in tall fescue this study was designed to examine the interaction of temperature and genotype on perloline accumulation, crude protein content, and total water soluble carbohydrates (WSC) on four selected tall fescue genotypes.

MATERIALS AND METHODS

Genotypes were selected from a previous study (23) based on average perloline content when grown at two locations, Oregon and Missouri. Genotypes 32 and 35 were selected from parents of a diallel used in the earlier study and ranked low and high, respectively, for perloline content. Genotypes 19 and 21 also exhibited low and high perloline levels, respectively, and are two of the eight parent clones of the cultivar 'Fawn.' The four genotypes were lifted from the field on June 9, 1977, increased vegetatively and established in 15 cm diameter pots using a soil mixture of 1/3 peat to 2/3 soil. On July 12, the plants were placed at random in control climate chambers. An average of three adjacent pots was used for each of four observations per genotype per temperature and harvest treatment. Growth chambers were calibrated for 15°C, 20°C, and 30°C with 12 hours of mixed fluorescent-incandescent illumination providing 350-400 μE in $^{-2}sec^{-1}$. In each case, the temperature was lowered 6°C to coincide with the 12 hour dark period. A modified-Hoaglands nutrient solution supplied by sub-irrigation was changed every 14 days. Forage samples were collected to within 5 cm of tiller stem bases following 28 days regrowth on August 9, September 6, and October 4 except for the third harvest of plants in the 30° chambers. In this case, the plants were destroyed due

to a chamber malfunction. In an effort to obtain plant material for this case, plants in the 20°C chamber were treated with 30°C for 28 days following the third harvest and then harvested on November 2, 1977.

Harvested plant tissue was microwaved in an oven for 1 minute, air dried in a convection oven for 48 hours at 65°C and ground to pass through a .5 mm screen.

Spectrophotometric analyses were used to measure water soluble carbohydrates, using anthrone reagent in H_2SO_4 as described by Yemm and Willis (25). High nitrate microkjeldahl digestions as described by Nelson and Sommers (18) were used for dtermining nitrogen content. Digested samples were distilled by the procedure of Brimner and Edwards (3) and and ammonical nitrogen determined by titration. Crude protein was estimated by multiplying percent nitrogen by 6.25. Perloline was measured using a technique of Schaffer et al. (21) when 50% ethanol extracted perloline is isolated on a cation exchange resin. Spectrofluorometric determinations were compared to a standard curve prepared from purified perloline. Perloline accumulation, crude protein content and water soluble carbohydrate data were analysed assuming a split-plot design with a factorial arrangement of treatments. Significant interaction terms were partitioned by genotype and examined by the regression approach.

RESULTS AND DISCUSSION

Significant harvest x temperature (P = .05), harvest x genotype (P = .05) and temperature x genotype (P = .01) interactions were observed for perloline level in this study. This supports earlier work showing significant genotype x environment interactions for perloline content (22) and its reported variation among cultivars (7, 13). Partitioning of the harvest x genotype interaction into estimates of harvest differences within genotypes for perloline level show (Fig. 1A) a significant linear decrease between harvests for three out of four genotypes, with number 32 showing no significant difference between harvest dates. Figure 1B illustrates the partition of the temperature x harvest interaction and shows that perloline level for the 30°C treatment had a higher rate of decrease with each harvest than 15° or 20°C. Decreases in perloline level in the 30°C treatment then appear to have contributed most to the genotype performances illustrated in Fig. 1A. Since these plants were grown in pots they may have become root bound over time. Gentry et al. (13) found that perloline was produced in the roots of tall fescue seedlings and highest concentrations were found in the roots of rapidly growing vegetative pasture plants. The decrease in perloline level with each harvest might be related to a reduction in root growth or increased root

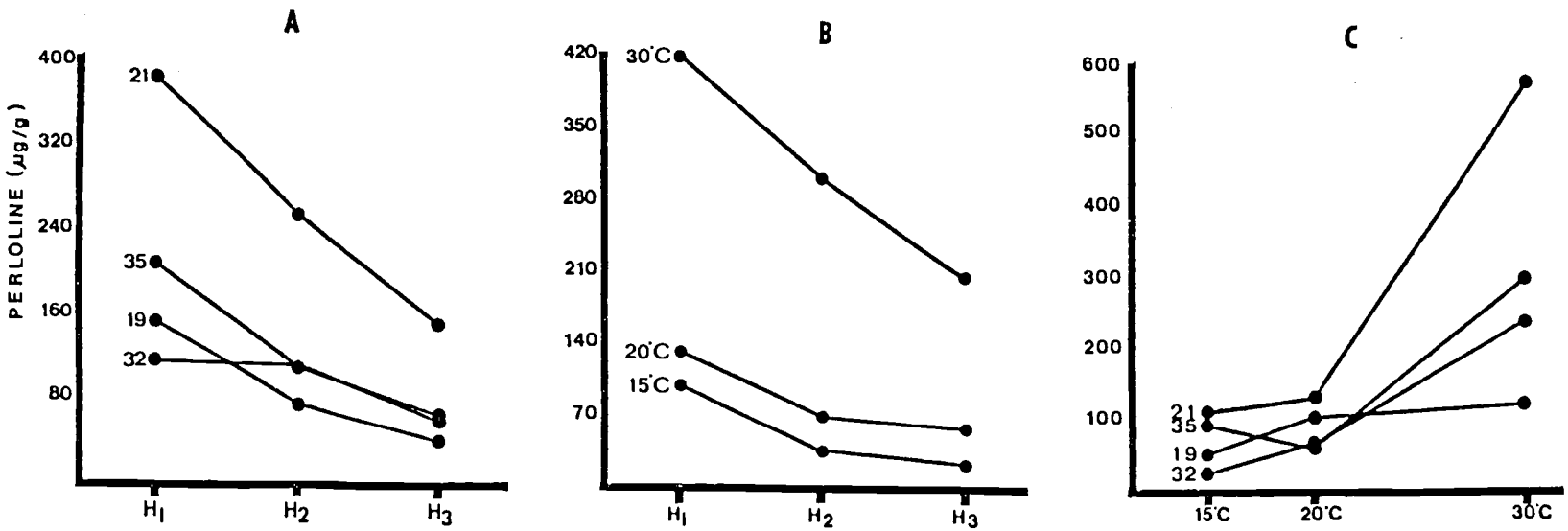

Figure 1. Changes in perloline with harvest date and temperature. Perloline level; (A) averaged over three temperatures, for each of four genotypes and three harvests, (B) averaged over four genotypes for each of three temperatures and three harvests, and (C) averaged over three harvests for each of four genotypes and three temperatures.

competition with time. In general, increasing temperature increased perloline content with a significant relationship between temperature and perloline of r = .587 (143 df). Linear regression analysis for perloline level for each genotype within temperature shows (Fig. 1C) that perloline level significantly increased linearly for all genotypes except for number 19 which is positive but not significant. Slope values and coefficients of determination for these regressions are given in Table 1, and indicate the increase in the measured component with each degree increase in temperature (°C). Genotype 35, rated as high for perloline in Watson's study (22) produced about half the level of perloline as genotype 21 at each temperature.

Miles (17) reported significant genotype x environment interaction for nitrogen content in tall fescue forage. The significant (P = .01) harvest x temperature and temperature x genotype interactions for crude protein reported here are in agreement with Miles. Differences in crude protein content for 15°C and 20°C treatments between harvest dates were not significant (Fig. 2A1), but a significant decrease for the 30°C treatment was noted in the final harvest. This decrease is believed to be caused by moving the plant material from the 20°C treatment to 30°C after the third harvest as explained in materials and methods. In general, increased temperature increased crude protein content, with a significant correlation

Table 1. Regression coefficients (b_1, b_2) and coefficients of determination (r^2 or R^2) for perloline (PER), crude protein (CP) and total water soluble carbohydrates (WSC) respectively on temperature. b_1 with r^2 indicates a linear relationship whereas b_1 and b_2 with R^2 indicates a quadratic relationship (n = 9).

	PER on Temperature		CP on Temperature			WSC on Temperature		
Genotype	b1	r^2	b1	b2	r^2	b1	b2	R^2
G_{19}	4.36	.215	.272	--	.539	-4.968	.091	.934
G_{21}	34.08	.743	.477	--	.789	-1.157	--	.951*
G_{32}	14.715	.955	.154	--	.420	-3.190	.061	.914
G_{35}	17.195	.684	2.647	-.064	1.00**	-5.105	.100	.676

*Coefficient of determination for linear relationship.

**Coefficient of determination for quadratic relationship.

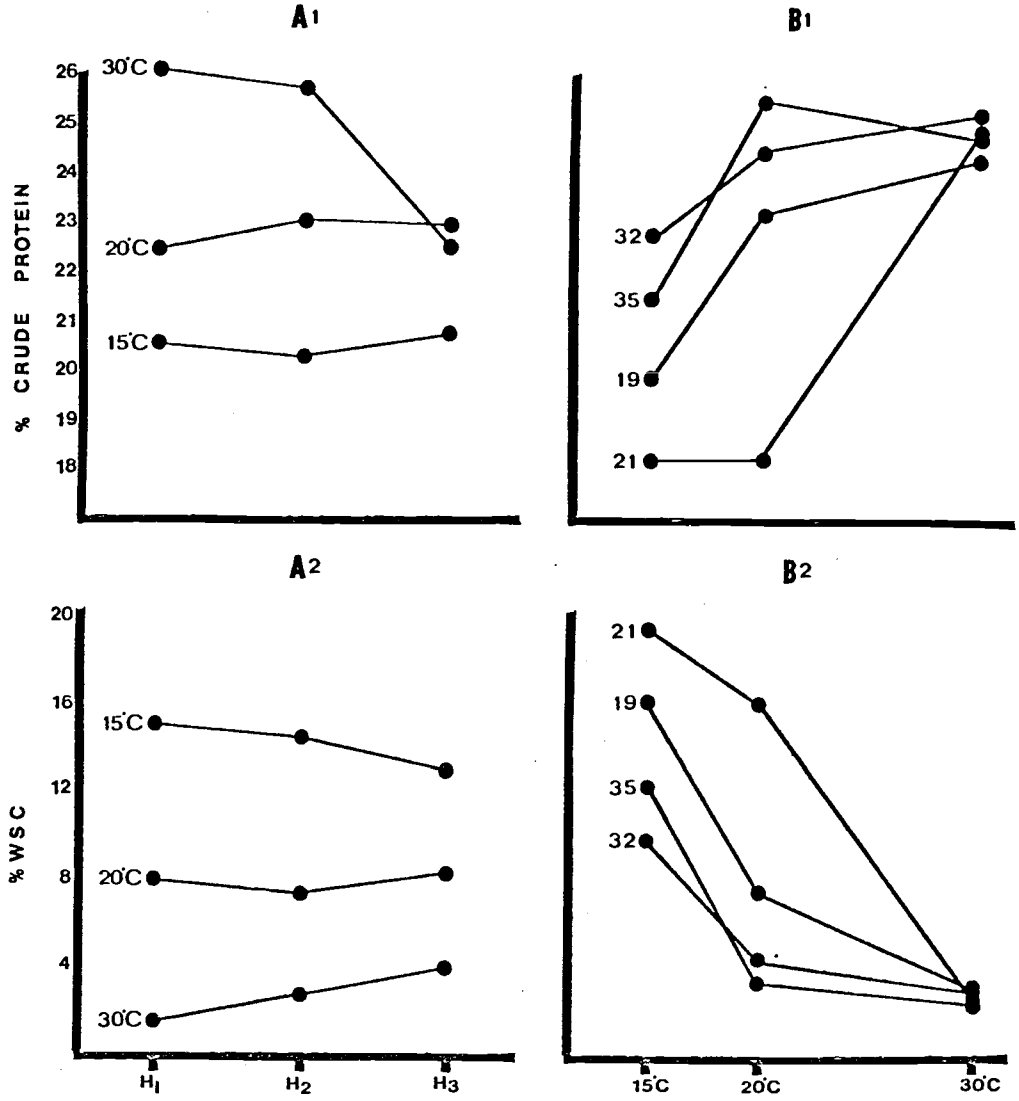

Figure 2. Crude protein; (A_1) averaged over four genotypes, for each of three temperatures and three harvests, and (B_1) averaged over three harvests, for each of four genotypes and three temperatures. Total water soluble carbohydrates (WSC); (A_2) averaged over four genotypes, for each of three temperatures and three harvests, and (B_2) averaged over three harvests, for each of four genotypes and three temperatures.

coefficient of r = .550 (143 df). Figure 2B1 shows genotype performance at each temperature, where genotypes 19, 21, and 32 are significantly linear, and 35 shows a non-linear relationship indicating an optimum for crude protein between 20°C and 30°C. Slope values from the regression analyses are in Table 1. Smith (20) found no significant differences between average tall fescue foliage crude protein for 20/10°C and 30/20°C treatments. The difference observed in this study may be due to the type of materials used and the frequency of harvests. Smith used a local tall fescue cultivar, 'Kentucky 31,' and harvested every 14 days where genotypes harvested every 28 days were used here.

Temperature has been shown to influence the accumulation of nonstructural carbohydrates in temperate origin grasses (20). With respect to WSC there were significant (P = .01) harvest x temperature and genotype x temperature interactions. WSC levels decreased linearly from harvest 1 to harvest 3 in the 15°C treatment (Fig. 2A2), showed no significant differences in the 20°C treatment and in the 30°C treatment increased linearly and significantly with each harvest. These relationships may indicate an effect due to conditioning of the plant materials to the different treatments over time. Smith (20) found a negative relationship between total water soluble carbohydrates and temperature in tall fescue. WSC levels, here, decreased (Fig. 2B2)

with increasing temperature. All genotypes showed decreasing WSC levels in a non-linear manner with largest differences between 15°C and 20°C, except genotype 21, which shows a significant linear decrease. Slope values from the regression analyses are in Table 1. The higher levels of WSC at lower temperatures seems to indicate WSC in excess of the needs of the plants for growth and maintenance.

Correlation coefficients between levels of perloline, crude protein, and WSC for each genotype are given in Table 2. Bennett (2) reported nitrogen content of perennial ryegrass to be positively associated with perloline. Crude protein content is based on the determination of both organic and inorganic nitrogen in the tissue. Perloline contains nitrogen, if derived from tryptamine and, therefore, must be part of the crude protein estimate. Here, perloline and crude protein are also positively associated, but only genotype 32 shows a significant association. The lack of significance appears to be caused by the decrease in perloline levels with each harvest, when crude protein levels remained relatively stable. Genotype 32, as noted, showed no significant change in perloline levels with each harvest.

Genotype ranking, based on average perloline level, is not the same as one based on average crude protein content. Those genotypes high in mean crude protein are not

Table 2. Simple correlation coefficients between perloline (PER), crude protein (CP) and total water soluble carbohydrates (WSC) for each genotype (n = 9).

Genotype	Per. vs CP	Per. vs WSC	CP vs WSC
G_{19}	.445	-.124	-.802*
G_{21}	.585	-.313	-.611
G_{32}	.812*	-.571	-.678
G_{35}	.555	-.267	-.366

*Significant at the .05 level.

necessarily high in perloline content. Genotype 21, the highest perloline producer was lowest in mean crude protein content, while genotypes 19 and 32, high in mean crude protein are rated low in perloline level. These observations suggest that perloline accumulation is not dependent upon crude protein levels. Positive associations (Table 2) suggest that perloline is part of the crude protein estimate.

If the increased formation of proteins from amino acids in response to temperature is accompanied by an increased energy requirement, then a carbohydrate pool, the source of such energy and structural units, may decrease or remain depressed. Crude protein is reported to be negatively correlated with percent soluble carbohydrates (4, 14). Correlation coefficients for crude protein and WSC for genotypes in this study are negative with only genotype 19 showing a statistically significant linear association. The association between perloline and WSC levels are also negative but not significant.

Bush et al. (6) found perloline inhibition of in vitro cellulose digestion and Fonnesbeck (12) reports soluble carbohydrates accounting for the major differences in digestibility. Differences in percent digestibility were not measured in this study but IVDMD is reported to decrease in tall fescue with increasing temperature (1, 20). Based on these reports and the data presented here, changes in forage quality due to seasonal temperature changes can be

expected. The measured components perloline, crude protein, and WSC are quality factors and the varied response by genotypes to temperature is important in assessing poor animal performance on newly developed cultivars. It is suggested that temperature screening will be valuable to the breeding program interested in selecting parent material for tall fescue forage quality improvement. Material with the genetic potential for being low or high in perloline level can best be screened at high temperatures, near 30°C, while crude protein and WSC can best be screened at lower temperatures, from 15° - 20°C. Further research is needed to determine specific environmental effects on these components and their relationship to animal performance.

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APPENDIX

Appendix Table 1. Perloline content (μg/g) of four tall fescue genotypes, arranged by treatment temperature, replication and harvest.

Temperature-Genotype	Harvest 1 Replications				Harvest 2 Replications				Harvest 3 Replications			
	1	2	3	4	1	2	3	4	1	2	3	4
15°C-19	27.58	102.52	72.42	104.92	72.70	23.50	43.78	28.48	13.00	12.75	14.75	18.00
15°C-21	97.34	202.33	234.73	172.43	84.58	44.20	43.32	24.49	33.14	28.48	14.51	52.70
15°C-32	32.30	19.86	19.36	28.46	13.13	18.62	16.75	11.88	12.00	16.00	14.50	14.25
15°C-35	104.90	221.56	32.32	118.80	14.50	20.50	31.87	51.42	14.76	12.00	14.50	17.25
20°C-19	111.29	284.75	155.07	122.55	109.99	107.42	62.47	91.24	41.65	37.40	30.60	39.95
20°C-21	267.84	245.12	104.98	227.50	87.49	128.72	89.96	76.05	75.67	76.51	75.24	52.70
20°C-32	52.29	77.38	110.00	26.78	45.04	44.62	69.25	62.88	42.09	41.65	105.02	47.61
20°C-35	98.80	53.54	86.24	70.76	53.97	50.97	45.89	37.82	32.30	37.41	39.96	61.21
30°C-19	429.27	99.96	127.45	163.72	51.00	73.53	54.38	141.27	71.41	57.81	82.49	46.75
30°C-21	765.00	408.00	1311.39	552.50	514.22	289.00	824.25	807.62	314.52	314.55	318.75	433.46
30°C-32	407.78	297.41	101.25	176.26	323.05	231.19	149.99	310.23	165.00	137.51	348.53	142.53
30°C-35	531.22	382.48	245.04	565.25	154.99	124.94	184.99	530.98	139.95	218.75	169.95	297.50

Appendix Table 2. Crude protein content (% dry weight) of four tall fescue genotypes, arranged by treatment temperature, replication and harvest.

Temperature-Genotype	Harvest 1 Replications				Harvest 2 Replications				Harvest 3 Replications			
	1	2	3	4	1	2	3	4	1	2	3	4
15°C-19	17.63	20.38	19.56	21.19	19.63	18.19	19.25	19.94	22.06	21.50	20.75	16.69
15°C-21	18.13	16.81	16.81	17.75	16.31	17.88	18.38	17.88	17.75	17.56	22.69	19.25
15°C-32	21.63	26.63	24.13	22.25	24.06	20.38	23.13	22.75	21.81	23.94	22.38	17.94
15°C-35	21.25	22.13	20.75	19.81	19.75	21.88	21.19	22.25	16.81	20.69	25.19	24.38
20°C-19	23.00	22.06	22.88	22.88	23.75	23.69	25.63	22.38	22.31	23.00	23.44	22.38
20°C-21	16.50	18.56	17.88	19.06	17.69	19.88	17.06	17.94	18.88	19.19	17.38	17.81
20°C-32	23.56	22.81	24.25	24.94	25.63	26.56	22.50	23.44	25.13	25.31	24.31	24.81
20°C-35	27.50	28.31	22.50	22.19	24.94	27.38	23.50	26.25	24.44	27.31	24.06	26.44
30°C-19	26.06	27.06	26.38	25.06	27.06	25.19	24.56	24.25	21.31	21.44	20.88	21.25
30°C-21	25.44	26.63	25.94	25.06	25.38	26.63	26.75	26.75	22.88	22.38	21.63	22.06
30°C-32	28.13	26.69	26.13	22.25	27.25	24.50	25.50	25.75	23.93	21.62	23.00	23.65
30°C-35	25.88	25.50	26.50	24.56	25.38	25.38	25.06	23.94	22.75	22.87	23.68	24.56

Appendix Table 3. Total water soluble carbohydrates (% dry weight) of four tall fescue genotypes arranged by treatment temperature, replication and harvest.

Temperature-Genotype	Harvest 1 Replications				Harvest 2 Replications				Harvest 3 Replications			
	1	2	3	4	1	2	3	4	1	2	3	4
15°C-19	19.76	19.29	16.70	13.87	19.27	16.92	15.56	17.72	12.27	12.43	14.33	15.88
15°C-21	21.63	19.96	23.72	19.82	21.71	20.30	18.10	20.17	21.25	18.24	12.22	16.71
15°C-32	13.91	7.36	9.32	9.66	8.26	13.96	5.31	8.56	9.85	9.41	8.48	12.57
15°C-35	14.66	8.89	15.38	9.90	16.13	9.26	10.55	9.87	16.89	11.07	8.42	8.61
20°C-19	6.49	10.35	10.28	8.02	7.25	6.61	4.30	7.38	7.27	7.92	7.49	6.14
20°C-21	20.17	14.59	11.87	13.61	19.87	11.93	19.49	14.26	18.99	15.26	15.91	17.03
20°C-32	4.92	7.28	3.13	4.38	3.61	2.37	3.71	4.25	3.46	5.46	5.02	4.96
20°C-35	2.73	1.72	2.97	4.39	2.40	2.16	4.00	2.77	4.82	4.04	4.18	4.36
30°C-19	1.02	0.96	0.12	1.38	2.88	2.91	3.27	2.45	5.31	7.02	4.76	4.86
30°C-21	1.11	1.23	0.71	1.46	3.23	2.92	2.92	2.62	3.65	4.32	3.57	3.60
30°C-32	1.97	2.05	1.22	2.07	2.86	2.78	1.74	2.36	3.47	5.09	3.20	4.63
30°C-35	2.02	1.24	1.62	1.92	2.62	2.61	2.27	2.87	2.59	2.48	2.49	2.83

Appendix Figure 4. Analysis of variance for perloline content (µg/g) of four tall fescue genotypes using a split-plot analysis with harvests as subplots.

Source	df	Mean Square
Treatments (T)	2	904529.44**
Genotypes (G)	3	223283.56**
T x G	6	123024.66**
Replications/GT	36	15202.31
Harvests (H)	2	183382.38**
T x H	4	21837.00*
G x H	6	24014.97*
T x G x H	12	7339.14
Error	36	8093.00

*,**Significant at the 5% and 1% levels of probability, respectively.

Appendix Table 5. Analysis of variance for crude protein content (% dry weight) of four tall fescue genotypes using a split-plot analysis with harvests as subplots.

Source	df	Mean Square
Temperatures (T)	2	218.32**
Genotypes (G)	3	104.00**
T x G	6	33.93**
Replications/GT	36	2.54
Harvests (H)	2	13.28**
T x H	4	24.22**
G x H	6	1.46
T x G x H	12	2.23
Error	72	2.14

**Significant at the 1% level of probability.

Appendix Table 6. Analysis of variance for total water soluble carbohydrates (% dry weight) of four tall fescue genotypes using a split-plot analysis with harvests as subplots.

Source	df	Mean Square
Temperatures (T)	2	1610.04**
Genotypes (G)	3	401.77**
T x G	6	116.53**
Replications/GT	36	5.92
Harvests (H)	2	1.14
T x H	4	25.10**
G x H	6	2.16
G x T x H	12	5.15
Error	72	2.84

**Significant at the 1% level of probability.