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Resistance of Multiple Diploid and Tetraploid Perennial Ryegrass (*Lolium perenne* L.) Varieties to Three Projected Drought Scenarios for the UK in 2080

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Abstract: Forage plants underpin the livestock industry. Selective breeding, including polyploidization, where genome size is increased by whole genome duplication, changes the productivity and stress tolerance of new varieties. We conducted a growth chamber experiment to investigate the likely responses of *Lolium perenne* L. to drought, testing four diploid and four tetraploid varieties. We simulated projected spring and summer temperatures for the South-West of England in 2080, applying three projected rainfall scenarios, which varied in drought severity. Drought caused a reduction in productivity, but there was substantial variation between varieties (up to 82%), with the optimal variety changing depending on drought severity. Across three harvests, productivity declined by 43% and 27% (dry biomass) for the severe and likely drought scenarios, respectively. In the final harvest, tetraploids exhibited a greater biomass under severe drought, whereas diploids had a greater biomass under the current rainfall and likely drought scenarios. Longer stomata were observed in tetraploids; however, stomatal conductance was not significantly different between ploidy levels. Trait selection will be important for future drought adaptation. Local climate projections will need to be consulted when selecting *L. perenne* varieties to tolerate the spatially variable reductions in future rainfall.

Keywords: biomass; drought tolerance; forage; nutrition; polyploidy; productivity; traits

1. Introduction

Plant breeding, including polyploidization (whole genome duplication), has sought to increase forage plant yields, nutritive values, and seed set. In the future, forage plants are very likely to be growing in different conditions than they are today. New varieties with different traits may become more valuable because rainfall has been projected to decline across many regions, strongly linked to anthropogenic greenhouse gas emissions [1]. Droughts may lead to a decline in forage productivity (defined as the rate of production of new biomass over a defined time period) and different varieties may be better adapted to future growing conditions [2]. Tetraploid perennial ryegrass varieties have been marketed as offering superior characteristics, including being more tolerant of drought; however, experiments comparing diploid and tetraploid varieties have provided mixed results. Total plant biomass was compared for different ploidy levels of the same variety of *L. perenne* and no significant differences in response to reduced soil moisture or severe defoliation were detected [3]. In field trials, variety tended to have a larger impact on root dry matter than ploidy, and under a more intense defoliation regime, ploidy resulted in no significant differences in root dry matter [4]. There are several other traits which may modulate drought resistance, including leaf size, crown diameter, plant height, leaf:stem ratio, and root architecture [5].

We sought to test the performance of eight different varieties (four diploid and four tetraploid) of the common forage grass, *L. perenne*, to varying drought severity under three rainfall scenarios and under temperature and humidity values predicted for SW England in 2080. This region contains the greatest density of livestock in the UK and may also be exposed to the most severe climate change [6]. *L. perenne* has a near-global distribution and is particularly common across Australasia, Europe, North and South America, and parts of Africa and Asia [7]. We hypothesized that (1) there would be a general decline in productivity under drought conditions; (2) there would be considerable variation in drought tolerance between varieties, but tetraploids would be more resistant to drought than diploids; and (3) that increased stomatal length and rates of conductance would influence drought tolerance.

2. Materials and Methods

We investigated eight *L. perenne* varieties, with four being intermediate flowering varieties (two diploid, two tetraploid) and four being late flowering varieties (two diploid, two tetraploid). The varieties have been anonymized in this manuscript on the request of the supplier. The intermediate varieties typically flower in the second half of May in the UK and late varieties typically flower in the first two weeks of June. The eight varieties were germinated in petri dishes from seed and following germination (17 days post-sowing), individuals were transferred to John Innes No. 1 compost. We planted 480 individuals (3 rainfall treatments × 8 varieties × 20 replicates) in multi-cell trays in groups of 10, which were placed in a HPP750 climate chamber (Memmert GmbH and Co: Germany). We positioned trays using a stratified random design and re-positioned them every two weeks to account for variation within the chamber. We simulated projected spring and summer conditions using two-hourly mean temperature and humidity values for the South-West of England [8], uplifted by the most likely scenario for 2080 (+3.9 °C) [6].

Seedlings were watered following the current rainfall regime for two months using April 2080 and May 2080 temperature and humidity settings. After two months, three rainfall treatments were applied: (1) current rainfall, (2) mean (−23%) projected decline for 2080 (hereafter: likely drought), and (3) maximum (−49%) projected decline for 2080 (hereafter: severe drought), and we switched to our June, July, and August 2080 temperature and humidity settings. This is in line with our current understanding of an anticipated summer, but not spring, drought for SW England in 2080 [6]. The amount of water applied was based on mean regional weather data (April–August), scaled to the surface area of the soil for each plant and then reduced by the proportions listed above. Watering volumes were reduced to 20% of the observed rainfall to take into account the reduced soil depth of the trays (7 mm), which is approximately one fifth of the rooting depth of *L. perenne* (c. 35 mm) [9,10] (see Appendix A, Table A1 for calculation).

We carried out three harvests, removing a third of the individuals after three, four, and five months. This was designed to simulate a three-cut strategy employed by many pastoral farmers. We measured fresh biomass (the above-ground mass of individual plants), dry biomass (the mass of the same individuals dried for 72 hours in a drying cabinet), and the proportion of dry matter (DM, 100 minus relative water content) at each time point. We also concurrently grew seedlings in ambient conditions in a glasshouse (RBG Kew) and measured stomatal length and stomatal conductance for all varieties. Stomatal conductance was measured at three time points during the study by randomly selecting fully expanded leaves from three plants from each variety and using a SC-1 Leaf Porometer (Labcell Ltd, Alton, UK). Stomatal length measurements were determined using a leaf scrape method to isolate the upper epidermis from each of the leaves. Leaf peels were stained by immersion in an aqueous solution of toluidine blue (0.1% *w/v*). De-stained leaf peels were mounted on slides with glycerol and imaged using a light microscope and digital camera. Stomata length measurements were made in ImageJ, using a stage micrometer to provide size calibrations [11].

Data were analyzed by analysis of variance (ANOVA) tests and post-hoc comparisons were done using Tukey's honest significant difference (HSD) tests. Separate ANOVA tests were carried out for variety identity and ploidy/timing because ploidy and timing are a subset of the varieties. We also tested for treatment effects and interactions between treatments for ploidy, timing, and drought regime across all biomass harvests using generalized linear models (GLM). All statistical analyses were carried out using the statistical software, R (v3.4.1) [12].

3. Results

Mean fresh biomass (−39%), dry biomass (−27%), and plant water content (−9%) declined under the likely drought scenario across all three harvests when compared with the current rainfall control (Table 1). In addition, mean fresh biomass (−58%), dry biomass (−43%), and plant water content (−22%) declined to a greater extent under the severe drought scenario, again across all three harvests compared to the control. Different varieties performed better than others and the optimal variety varied between the rainfall treatments ($F = 2.1$, $df = 7$, $p < 0.05$). Variety8 produced the most dry biomass under the current rainfall scenario (82% more than the lowest) and Variety6 the most dry biomass under the severe drought scenario (58% more than the lowest). Variety2 and Variety6 produced the most dry biomass under the likely drought scenario (Figure 1).

Table 1. ANOVA results for live biomass, dry biomass, and proportion of dry biomass (DM) across each of the monthly harvests. Ploidy, rainfall treatments, and timing were included as potential explanatory variables. Degrees of freedom (df) were equal for all three of the biomass metrics.

			Live Biomass (g)		**Dry Biomass (g)**		**DM (%)**	
		df	**F**	***p***	**F**	***p***	**F**	***p***
Harvest 1	Ploidy	1	0.40	0.54	0.01	0.90	0.001	0.97
	Rainfall	2	38.80	<0.001	4.40	<0.05	30.20	<0.001
	Timing	1	3.20	0.07	6.60	<0.05	0.05	0.82
Harvest 2	Ploidy	1	0.40	0.50	0.30	0.60	2.40	0.10
	Rainfall	2	34.50	<0.001	5.10	<0.01	7.00	<0.01
	Timing	1	2.50	0.10	0.60	0.50	0.30	0.60
Harvest 3	Ploidy	1	6.40	<0.05	4.80	<0.05	2.50	0.10
	Rainfall	2	62.00	<0.001	63.10	<0.001	7.40	<0.001
	Timing	1	0.40	0.50	0.001	0.90	1.90	0.20

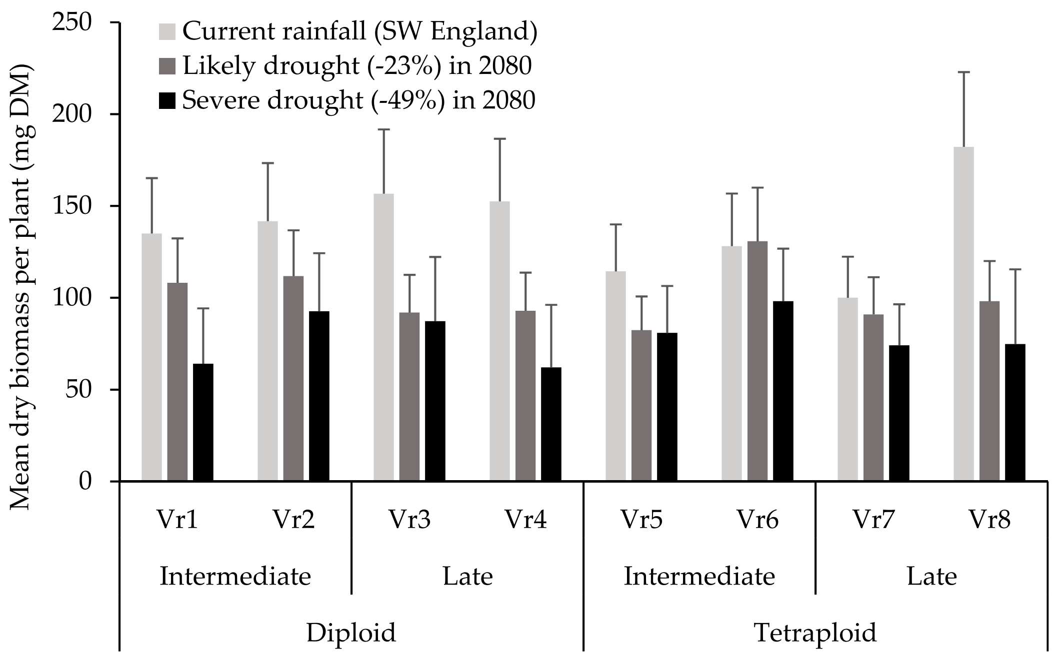

Figure 1. Mean dry biomass (with standard error bars) produced by each variety (Vr) averaged across all three harvests. Varieties are partitioned by ploidy (diploid or tetraploid) and by flowering time (intermediate or late).

There were no significant differences in productivity between ploidy levels for the first two harvests (Figure 2, Table 1). However, in harvest 3, diploids produced more fresh and dry biomass under the current rainfall scenario (+25% and +28%, respectively) and likely drought scenario (+13% and +9%, respectively), whilst tetraploids produced more dry biomass (+13%) with a lower water content (−9%) under the severe drought scenario. Tetraploids were generally more resistant to severe drought in harvest 3; however, under the other scenarios, the diploids generally produced more biomass, even under the most likely drought scenario. No effect of variety timing on resistance to drought was observed, although intermediate varieties produced a mean of 25% more dry biomass than late varieties in harvest 1. The GLM analysis confirmed that the significant ANOVA results for the rainfall treatments were robust across all harvests; however, no other significant treatment effects were detected. In the case of ploidy, this may have been because significant effects of ploidy in harvest 3 may have been obscured by non-significant effects in harvests 1 and 2. No interactions between treatments were detected (all $p > 0.05$).

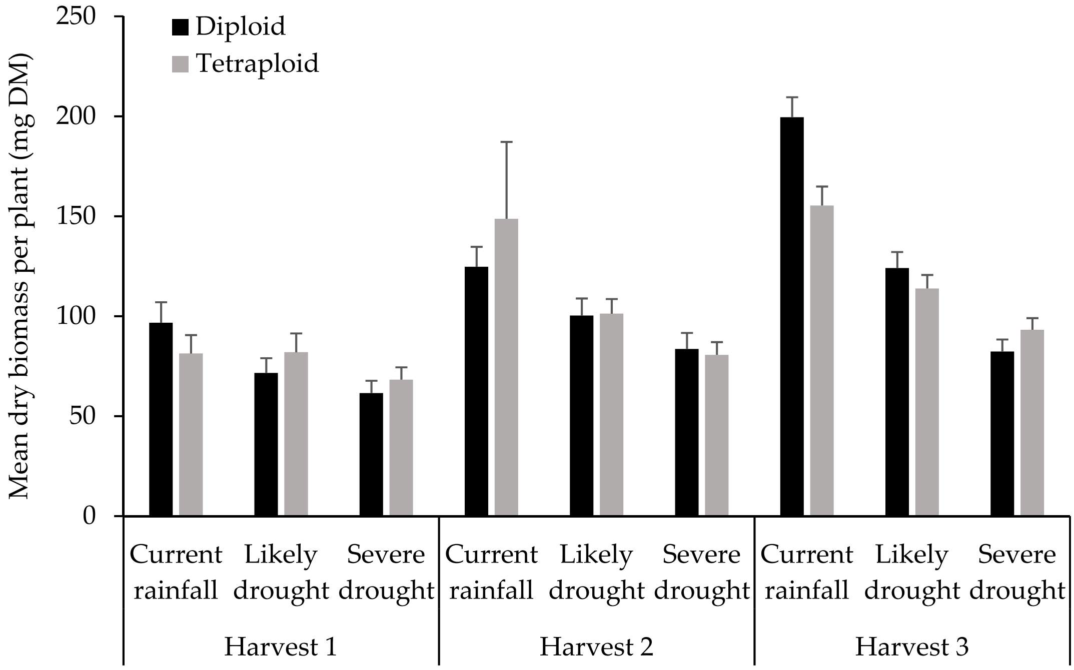

Figure 2. Mean dry biomass (with standard error bars) produced by diploids and tetraploids for all three harvests.

Mean stomatal length varied 1.5-fold between varieties (F = 173.7, df = 7, $p < 0.001$) and stomatal conductance varied 1.4-fold between varieties (Table 2), although the latter was not significant (F = 1.2, df = 7, $p = 0.33$). On average, tetraploid stomata were 24% longer than diploids (F = 173.6, df = 1, $p < 0.001$) and the mean rate of conductance was 10% faster, although the latter was marginally not significant (F = 3.9, df = 1, $p = 0.06$). No correlation was found between plant productivity under drought conditions and stomatal length or conductivity (both $p > 0.05$).

Table 2. Mean ± standard error (SE) stomatal size and conductance for each variety (Vr) and ploidy level.

	Stomatal Length (µm)		Stomatal Conductance (mmol m^{-2} s^{-1})	
	Mean	**SE**	**Mean**	**SE**
Vr1	26.52	0.29	46.62	11.61
Vr2	29.59	0.26	41.63	4.85
Vr3	33.29	0.85	36.13	5.04
Vr4	29.86	0.43	36.25	3.02
Vr5	35.67	0.39	48.79	8.81
Vr6	40.77	0.54	41.30	7.52
Vr7	34.20	0.43	38.78	4.74
Vr8	37.60	0.26	46.73	9.29
Diploid	30.67	0.40	40.15	3.30
Tetraploid	38.84	0.45	44.03	3.63

4. Discussion

Many regions are likely to experience more frequent droughts in the future, but the effects of reduced water availability on forage production and on the livestock industry have not yet been fully quantified. The declines in productivity of 43% and 27% (dry biomass) that were recorded for the severe and likely drought scenarios, respectively, would represent substantial declines in the amount of available forage for livestock if replicated on a wide scale in 2080. Declines in plant water content and a rising proportion of DM are also noteworthy under drought conditions, since additional water will need to be obtained from other sources by grazers. *L. perenne* is a very common species with a near global distribution, so the implications of these finding may be widespread [7].

The variation in drought tolerance between varieties (up to 82%) indicates that variety selection will be important for drought adaptation in the future. Additional complexity is added because the optimal variety changes depending on drought severity. The frequency and magnitude of projected changes to rainfall are likely to be spatially variable [1] and therefore, local projections need to be consulted when selecting varieties to cultivate to adapt to the changing growing conditions. This is consistent with previous studies highlighting that variation in traits between varieties of the same species is an important determinant of drought tolerance [3,13]. Comparisons of varieties are necessary for drought adaptation if a general reduction in the production of forage for livestock consuming *L. perenne* is to be avoided.

Tetraploids accrued a higher biomass under the severe drought scenario and diploid varieties generally produced the most biomass under the most likely drought scenario, but these differences were only detected in harvest 3. Tetraploids may offer advantages if severe drought occurs in 2080; however, variation between varieties and the likelihood of drought severity will need to be considered. Tetraploids had longer stomata, but the lack of a direct relationship between plant productivity and either stomatal length or conductivity indicated that any differences due to stomatal length were obscured by other factors. Traits other than polyploidy also confer drought tolerance; such as a shorter habit, early maturity, plasticity in leaf chemistry for osmotic control, and the capacity for prolonged stomatal closure which reduces evapotranspiration and may save water [14], whilst deeper, finer roots, may promote water acquisition [15]. The full suite of available drought tolerance traits, including polyploidy, will need to be considered for future forage plant breeding.

Recent work has shown that grasses can vary in terms of protein (2–36%), fiber (27–90%), minerals (2–19%), and lignin contents (1–21%), and these values are influenced by climate [16]. Future work should measure the effects of different aspects of rainfall variability (including more frequent drought and flooding) on the productivity and nutritive values of different forage plant species and varieties using growth chambers and field trials over longer timeframes. The drought tolerance of individual varieties, specific traits, or ploidy levels could be more or less resistant to drought over time. In the case of ploidy, for example, it is unclear whether the performance improves or declines after year one [17].

Furthermore, climate change is a multi-factor phenomenon and the effects of rising carbon dioxide concentrations, variable temperatures, and other biogeochemical changes were not included here. Nevertheless, our study represents an important step forward by providing evidence that future forage grass productivity and plant water content are likely to decline under summer droughts. Drought tolerance traits, as well as ploidy level per se, should be considered in the breeding and selection of future *L. perenne* varieties and these traits should be tailored for local drought severity.

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Appendix A

Table A1. Calculation for fortnightly watering volume for climate treatments. Volumes were reduced proportionally to account for plants removed during harvests.

Parameter	Value
Cell dimensions (mm)	38 × 38
Cell area (mm^2)	1444
Current rainfall [1] (mm)	7
Volume of rainfall per cell (mm^3)	10,108
Cell number per tray	40
Total rainfall per tray (mm^3)	404,320
Total volume per tray (ml)—current rainfall	404
Total volume per tray (ml)—likely drought	303
Total volume per tray (ml)—severe drought	202

[1] Mean current rainfall for SW England (April–August) is 2.5 mm per day [8], divided by five to account for reduced rooting depth (see main text).

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