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Effects of plant architecture and drought stress level on lucerne forage quality

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Abstract

Breeding for enhanced quality in lucerne (*Medicago sativa*) frequently involves selection for higher leaf-to-stem ratio, multifoliolate leaves or short-internode stems. Three populations selected for such alternative morphologies and a reference cultivar were evaluated for forage yield, leaf-to-stem ratio and protein and fibre concentrations in leaves, stems and whole plants. Four managed environments were obtained by combining two stress levels (moderate or nil) with two sowing times. The population selected for high leaf-to-stem ratio, as well as the short-internode population, had highest leaf-to-stem ratio (1.27) across six harvests in two non-stress environments. The latter population had higher stem protein (12.9%) and lower stem neutral-detergent fibre (NDF) concentration (58.7%) than other populations. The multifoliolate population had intermediate quality, showing low expression of the multifoliolate trait (14.0% across four environments), particularly under stress (10.5%). The autumn-sown, fully irrigated environment had, on average, highest dry-matter yield (4.19 t ha^{-1}) and lowest leaf-to-stem ratio (0.74). Drought-stressed environments had lower plant NDF (−12.3% on average) and leaf protein (−9.7%), and higher stem protein (+8.6%) than fully irrigated environments. The results suggested that environmental effects might have greater impact on quality than genetic effects, even for a population set including material selected for quality-driven morphology.

KEYWORDS
alfalfa, drought, internode length, leaf-to-stem ratio, *medicago sativa*, multifoliolate leaves

1 | INTRODUCTION

Lucerne (alias alfalfa, *Medicago sativa* L.) forage is renowned for its high nutritive value. Nonetheless, improved forage quality is increasingly important as a breeding goal, to match the requirements of genetically improved, increasingly high-performing dairy and beef cattle (Annicchiarico, Barrett, Brummer, Julier, & Marshall, 2015; Émile, Mauries, Allard, & Guy, 1997).

The nutritive value of lucerne depends mainly on the contents of crude protein (CP) and fibre fractions associated with higher digestibility, such as neutral-detergent fibre (NDF), acid-detergent fibre (ADF) and acid-detergent lignin (ADL). Appreciable genetic differences for these traits have been inconsistently reported (Coors, Lowe, & Murphy, 1986; Lenssen, Sorensen, Posler, & Harbers, 1991; Rotili, Gnocchi, Scotti, & Zannone, 1991; Tremblay, Bélanger, McRae, & Michaud, 2002), and selection progress for improved forage quality has generally been limited (Annicchiarico et al., 2015). Cultivars claimed for specifically enhanced quality attributes are few (Hall, Smiles, & Dickerson, 2000; Huset, Schnebbe, Kugler, & Peterson, 1991), and quality-improved germplasm has not always exhibited higher nutritive value than other cultivars (Martin & Sheaffer, 1998). Other factors are known to interact with the cultivar in determining the nutritive value of lucerne, thus hindering possible genetic effects. Plant developmental stage at harvest is a major factor affecting the

forage intake and digestibility and the protein concentration (Julier & Huyghe, 1997; Marten, Buxton, & Barnes, 1988; Palmonari, Fustini, Canestrari, Grilli, & Formigoni, 2014). Other factors may influence forage quality, with cultivars showing complex interactions with growing environment, harvest date and stand age (Julier, Huyghe, & Ecalle, 2000; Sheaffer et al., 1998). Within-cultivar variation for quality traits may be large and usuallys the offset differences between cultivars (Annicchiarico, 2007a; Julier et al., 2000).

Higher nutritive value of lucerne appears related to greater stem digestibility, greater proportion of leaves or both (Buxton & Hornstein, 1986; Hall et al., 2000; Jung & Lamb, 2006; Lemaire & Allirand, 1993; Sheaffer et al., 2000). As a consequence, breeding for enhanced quality has frequently targeted morphological traits that ensure higher leaf biomass in the lucerne canopy, such as increased leaf-to-stem ratio (Marten et al., 1988), multifoliolate leaves (Volenec & Cherney, 1990) or stems with shorter internodes (Scotti et al., 2007). Selection for high leaf-to-stem ratio can be an appropriate breeding objective to improve forage quality, owing to its close association with forage digestibility and intake (Kephart, Buxton, & Hill, 1990; Lemaire & Allirand, 1993), its moderate to high narrow-sense heritability (Annicchiarico, 2015; Guines, Julier, Ecalle, & Huyghe, 2002) and its low to nil genetic correlation with forage yielding ability (Annicchiarico, 2015; Julier et al., 2000).

Higher summer temperatures and evapotranspiration, lower and less dependable rainfall, and lower availability of irrigation water are expected in most lucerne growing regions due to climate change. Reduced growth caused by water deficits can result in a reduction in fibre concentrations and an increase in digestibility of lucerne (Deetz, Jung, & Buxton, 1996; Halim, Buxton, Hattendorf, & Carlson, 1990; Lemaire, Durand, & Lila, 1989). However, effect of drought on protein content is inconsistent (Carter & Sheaffer, 1983; Halim et al., 1990; Lemaire et al., 1989; Petit, Pesant, Barnett, Mason, & Dionne, 1992). Breeding for specific adaptation to moisture-favourable or unfavourable conditions can be a valuable strategy (Annicchiarico, 2007b) that requires, however, knowledge on forage quality traits of greater specific importance in a given condition.

Our objective was to assess the effect of plant architectures deriving from different breeding strategies (multifoliolate leaves, stems with shorter internodes or higher leaf-to-stem ratio) on lucerne forage quality under contrasting levels of water availability.

2 | MATERIALS AND METHODS

Our study focused on three lucerne populations representing different plant morphological types for potentially enhancing lucerne forage quality, namely (i) the experimental population Miranda, selected for the presence of multifoliolate leaves; (ii) a short-internode selection (SI-Sel) from a divergent selection programme for stem internode length (Scotti et al., 2007); and (iii) the experimental population MSI030, derived from the simultaneous selection for high forage yield and high leaf-to-stem ratio as described by Annicchiarico (2015). The commercial variety Prosementi, characterized by wide adaptation across contrasting drought stress levels in northern Italy (Annicchiarico & Piano, 2005), acted as a high-yielding control not subjected to specific forage quality improvement. All four populations had NAAIC cold-season dormancy scores of around 6, suitable for lucerne growing in northern Italy environments.

The four populations were field grown during the years 2012 and 2013 in Lodi, northern Italy (45°19′N, 9°30′E; 81 m elevation), under a rainout shelter covered with polythene sheets and equipped with low-pressure sprinklers. The study involved two experiments corresponding to two sowing times, each including two managed environments with contrasting summer drought stress levels (Table 1). The soil was a sandy loam with subacid pH 6.5, low in organic matter (16.6 g kg^{-1}) and potassium (48.4 mg kg^{-1}), and intermediate in nitrogen (1.27 mg kg^{-1}) and phosphorus (41.5 mg kg^{-1}). Prior to both sowings, fertilizer was applied at

TABLE 1 Irrigation and main growth conditions of four managed environments (Env1 - 4) implemented in two consecutive experiments of lucerne evaluation under a field rainout shelter

	Experiment 1		Experiment 2	
	Env1	Env2	Env3	Env4
Sowing season (date)	Spring (22 February 2012)	Spring (22 February 2012)	Autumn (15 September 2012)	Autumn (15 September 2012)
Total irrigation water (mm)	650	310	760	460
Days of irrigation withholding (dates)	0	59 (23 June – 20 August 2012)	0	103 (15 May–25 August 2013)
Average maximum daily temp. hottest month (°C)	33.8	33.8	32.7	32.7
Water deficit during the cropping period (mm)[a]	0	332	44	344
Number of harvests (dates)	4 (18 June, 10 July, 9 August, 11 September 2012)	3 (18 June, 10 July, 11 September 2012)	5 (14 May, 20 June, 22 July, 26 August, 20 September 2013)	3 (14 May, 20 June, 20 September 2013)

[a]As the difference between estimated long-term potential evapotranspiration and irrigation water applied.

50 kg ha^{-1} N, 150 kg ha^{-1} P_2O_5 and 150 kg ha^{-1} K_2O. Pest and disease control were not necessary and, based on field history, seed inoculation was also not required. Both experiments were conducted during one growing season. The combination of two sowing times × two stress levels resulted in four environments for germplasm evaluation.

The first sowing was on 22 February 2012, corresponding to a spring-to-summer crop cycle. Seeds were sown in polystyrene plug-trays, and the seedlings were field transplanted on 16 April 2012 in 1.08 m^2 plots (75 cm × 144 cm) with 10 rows each and with 18 plants spaced 7.5 cm apart between rows and 8.0 cm apart within rows.

The first experiment consisted of two managed environments, defined as Env1 and Env2. For Env1, no drought was imposed and a total irrigation of 650 mm was applied every 10–14 days from 17 April 2012 until the end of the experiment on 11 September 2012. For Env2, there were 59 days of no irrigation between 23 June 2012 and 20 August 2012. An irrigation total of 270 mm was applied to Env2 before the drought stress period and 40 mm after the stress period, concurrently with irrigation of Env1.

The second experiment was sown on 15 September 2012 as above, but corresponding to an autumn-to-summer crop cycle. Field transplanting was completed on 29 October 2012 with the same plot design as the first experiment. This experiment also consisted of two managed environments defined as Env3 and Env4. In Env3, there was no drought stress and a total of 700 mm irrigation was applied every 14–20 days between 15 March 2013 and 20 September 2013. For Env4, water was withheld for 103 days between 15 May 2013 and 25 August 2013. An irrigation of 250 and 150 mm was applied before and after stress imposition respectively. Env3 and Env4 did not differ for irrigation during the winter months after transplantation, which amounted to 60 mm.

Each managed environment of both experiments was designed as a randomized complete block experiment with three replications per population. The 12 plots (experimental units) were arranged in a row flanked on both sides by border plots. At both sowing times, the two environments were under the same rainout shelter and separated by an unplanted 5.5-m-wide strip to avoid any water from the irrigated environment inadvertently reaching the stressed plots. Mist drift from the irrigated to the stressed environment was prevented by placing a vertical polythene sheet across the unplanted space.

Daily temperatures were recorded in both experiments. Long-term monthly values of potential evapotranspiration (PET) were estimated from temperature values, as in Annicchiarico et al. (2011). A drought stress index was computed for each environment as the difference between long-term PET and water provided by irrigation during each field cropping period (Table 1).

Four harvests of aerial biomass were made in Env1 (Table 1) by mowing each plot at 5 cm height above the ground, excluding the two outer rows. We adopted a 5-cm cutting height as an acceptable compromise to maximize dry-matter (DM) yield and quality, with moderate lignification of the lowest harvested portion of the stems. Env2 was mown three times with the same procedure (Table 1). The first harvest of Env2 (18 June 2012) was made before any stress imposition. Populations were mown when about 75% of plants were at early flowering (first open flowers) as recommended by the hay industry in Italy to maximize lucerne forage quality. That resulted in up to 3 days difference in harvest dates among the populations in the summer harvests. Phenological differences were negligible in spring harvests. Differences in mean flowering time between the drought-stressed and unstressed environments at harvest 2 were significant ($p < .05$) but of little relevance in practical terms (1.3 day earlier flowering in Env2 compared to Env1; 1.5 day earlier flowering in Env4 than Env3 in the second experiment).

The irrigated Env3 was harvested five times and the drought-stressed Env4 three times (Table 1). The first harvest of Env4 (14 May 2013) was carried out before stress imposition. In the summer harvests of June and July, Miranda and SI-Sel were again mown with a delay of about 2–3 days compared to the other populations to ensure similar phenological stages at harvest.

The second harvest was carried out on 10 July 2012 for Env1 and Env2, and on 20 June 2013 for Env3 and Env4. For the drought-stressed environments Env2 and Env4, harvest occurred during the water withholding period (18 and 37 days after stress imposition respectively). At harvest, Env2 and Env4 had received 80 and 100 mm less irrigation than Env1 and Env3 respectively.

Data from the second harvest were used to compare the four environments and the four populations. For that harvest, DM yield of aerial tissues after oven drying at 60°C to constant weight, and leaf-to-stem ratio on a dry weight basis, were recorded on all plots. Leaf blades and petioles were retained as 'leaves', with the remaining aerial parts classified as 'stems' as in previous studies (e.g. Julier et al., 2000). Prior to the plot harvest, six main shoots from as many randomly sampled plants per plot were cut from the crown at ground level, and stem length, number of internodes per stem and mean internode length (averaged across all internodes) were immediately recorded on each shoot. The six subsamples per plot were averaged for statistical analyses. Stem length and number of internodes per stem were measured from the ground to the node immediately beneath the growing apex of the stem. In addition, the total number of multifoliolate leaves (those with more than three leaflets per leaf) on the six stems was counted and the percentage of multifoliolate leaves was computed.

Plot samples collected during the second harvest from the first two replications of each environment were used for chemical determinations of CP, NDF, ADF and ADL concentration, as well as NDF digestibility after 24 hr, which is an important parameter of forage quality (Oba & Allen, 1999). Chemical analyses were carried out separately on leaf and stem samples from each plot. Prior to chemical analysis, a random sample of leaves and stems from each plot was ground in a Cyclotec 1093 sampling mill (Foss Tecator AB, Höganäs, Sweden) through a 1-mm sieve. Based on the concentrations measured for leaves and stems and on the recorded leaf-to-stem ratio, an overall value for the whole herbage was also computed a posteriori for CP, fibre fractions and NDF digestibility.

Chemical analysis and in vitro fibre digestibility were carried out as described previously (Palmonari et al., 2014, 2016). Crude protein (AOAC 976.06, 984.13), amylase-treated ash-corrected NDF with addition of sodium sulphite (Mertens, 2002), ADF (AOAC 973.18) and ADL (AOAC 973.18) were analysed as described in Palmonari et al. (2014), with the addition of microfibre glass filters (1.5 μm; Whatman International Ltd, Maidstone, UK) to each crucible, as suggested by Raffrenato and Van Amburgh (2011). The NDF correction for ash residues is essential for forages such as lucerne that can have significant ash content.

In vitro NDF digestibility after 24 hr was performed using the Tilley and Terry modified technique (Robertson & Van Soest, 1981; Tilley & Terry, 1963). Rumen fluid was collected through rumen cannula from two lactating cows fed a hay-based diet, mixed and placed in a thermostatic bottle. Rumen contents were filtered through four layers of cheesecloth under constant O_2-free CO_2. Rumen fluid Ten millilitres were added to each 150-ml Erlenmeyer flask that had been placed in a heated (39.3°C) water bath under CO_2-positive pressure to ensure anaerobiosis. Samples of 0.5-g ground forage were weighed into each flask before the addition of 40 ml of the buffer described by Goering and Van Soest (1970). Each sample was analysed in two separate in vitro incubations, keeping the same sample preparation and donor cows for both samples. At the end of the fermentation, the content of each flask was analysed to determine the NDF content of the residue, and filtered through crucibles (40-μm porosity) with the addition of microfibre glass filters. The residues were then treated following the procedure described by Goering and Van Soest (1970) with the hot weight of the crucibles recorded after 3 hr drying in a forced-air oven (105°C). Ash correction was made after incineration of the residue at 495°C for 3 hr, followed by a second crucible hot weight. The digestibility was calculated as described in Equation 1:

$$\text{IVNDFD, \%NDF} = [1 - (\text{NDF}_r - \text{NDF}_b/\text{NDF}_i)] \times 100 \quad (1)$$

where NDF_r is the residual NDF, NDF_b is the blank correction, and NDF_i represents the initial NDF.

Dry-matter yield and leaf-to-stem ratio under favourable moisture conditions (Env1 and Env3) were investigated further for a more thorough characterization of the populations. The total DM yield per plot was computed by summing the values of DM yield recorded in the harvests 1–4. The mean leaf-to-stem ratio was computed as the average of the ratios recorded at harvests 2, 3 and 4 (summer) in Env1 and harvests 1, 2 and 5 (spring and summer) in Env3.

Yield, morphological and chemical data from the second harvest in the four environments were subjected to an analysis of variance (ANOVA) that included the fixed factors 'environment' and 'population' and the random factor 'block within environment', testing differences among environments and among populations, as well as their interaction. Hence, the joint analysis of the different environments was equivalent to the analysis of a variety trial performed in different sites (Snedecor & Cochran, 1989). A second ANOVA assessed the variation among populations for total DM yield and mean leaf-to-stem ratio across moisture-favourable environments. When the F test in an ANOVA was significant ($p < .05$), differences in environment or population mean values were compared by the least significant difference (LSD) test at $p < .05$. All statistical analyses were carried out using SAS software.

3 | RESULTS

Withholding irrigation in Env2 and Env4 resulted in little more than 300 mm water deficits (Table 1). Summer high temperatures also affected both experiments, with long spells of daily maximum temperatures exceeding 30°C (Table 1). On average, maximum temperatures in summer under the rainout shelter were about 1°C higher than in the surrounding fields.

Significant variation ($p < .01$) among environments was observed for DM yield, leaf-to-stem ratio, stem length, number of internodes per stem, mean internode length and percentage of multifoliolate leaves recorded in the second harvest (data not shown). At that harvest, the four populations differed ($p < .05$) for all characters except leaf-to-stem ratio, with a significant environment × population interaction ($p < .01$) only occurring for the percentage of multifoliolate leaves.

As expected, drought reduced the forage production in Env2 and Env4 compared to the moisture-favourable environments with the same sowing date (Table 2). Although both Env1 and Env3 were moisture-favourable treatments, Env3 had longer growing period than Env1 and had the highest mean DM yield, along with more numerous and longer stems with longer internodes than all other environments (Table 2). The numerous and longer stems of Env3, however, caused a distinctly lower leaf-to-stem ratio relative to the other environments (Table 2). Although there were only four data points, a trend towards an inverse relationship between environment DM yield and leaf-to-stem ratio ($r = 0.92$, $p < .08$) was evident. The proportion of multifoliolate leaves tended to be higher in the spring-sown environments, but only Env1 significantly differed from the others ($p < .05$), with a modest overall percentage of about 7% multifoliolate leaves (Table 2).

Only moderate differences among populations were found for mean DM yield across environments, with Prosementi and MSI030 yielding more than Miranda (Table 3). Higher yield by Prosementi and MSI030 was associated with a trend towards longer stems, with Miranda showing intermediate stem length, to which the lower number of internodes per stem might have contributed (Table 3), although there was no overall correlation between population stem length and number of internodes (data not shown). Consistent with their selection history, SI-Sel had significantly shorter internodes ($p < .05$) than other populations, and Miranda was the only population showing multifoliolate leaves (Table 3). The reduced internode length of SI-Sel contributed to its shorter stem length, given the positive association between the two traits ($r = 0.90$, $p < .10$). The environment × population interaction for the percentage of multifoliolate leaves resulted from higher values for Miranda under moisture-favourable conditions than under drought (17.5% across

TABLE 2 Mean and standard error (SE) values of lucerne dry matter and plant morphological traits recorded in the second harvest (early summer: 10 July 2012 for Env1 and Env2; 20 June 2013 for Env3 and Env4) from four managed environments (see Table 1 for environment description)

Environment	Dry matter (t ha^{-1})	Leaf-to-stem ratio[a]	Stem length (cm)	No. of internodes/stem	Internode length (cm)	Multifoliolate leaves (%)
Env1	2.61 b	0.99 a	47.8 b	7.87 b	6.16 b	7.1 a
Env2	2.03 c	1.02 a	39.0 c	6.40 c	6.21 b	3.7 b
Env3	4.19 a	0.74 b	76.1 a	11.47 a	6.71 a	1.6 c
Env4	1.93 c	0.95 a	42.1 c	7.00 c	6.13 b	1.3 c
$SE_{(df=11)}$	0.06	0.03	1.6	0.22	0.12	0.7
$LSD_{(p < .05)}$	0.18	0.08	4.8	0.64	0.35	2.0

In each column, mean values followed by different letters were different at $p < .05$ according to the respective least significant difference (LSD).
[a]Based on dry weight.

TABLE 3 Mean and standard error (SE) values of dry matter and plant morphological traits of four lucerne populations, recorded in the second harvest (early summer: 10 July 2012 for Env1 and Env2; 20 June 2013 for Env3 and Env4) over four managed environments, and quality selection criterion for each population

		Characters					
Population	Selection criterion	Dry matter (t ha^{-1})	Leaf-to-stem ratio[a]	Stem length (cm)	No. of internodes/stem	Internode length (cm)	Multifoliolate leaves (%)
MSI030	High forage yield and leaf-to-stem ratio	2.78 a	0.93 a	54.9 a	8.17 ab	6.75 a	0.0 b
Miranda	Multifoliolate leaves	2.55 b	0.93 a	49.9 ab	7.71 b	6.50 a	14.0 a
Short-internode selection (SI-Sel)	Short internodes	2.64 ab	0.94 a	46.0 b	8.43 a	5.50 b	0.0 b
Prosementi	Control variety	2.78 a	0.91 a	54.0 a	8.44 a	6.48 a	0.0 b
$SE_{(df=11)}$		0.06	0.03	1.6	0.22	0.12	0.7
$LSD_{(p < .05)}$		0.18	NS	4.8	0.64	0.35	2.0

In each column, mean values followed by different letters were different at $p < .05$ according to the respective least significant difference (LSD).
NS, not significant.
[a]Based on dry weight.

Env1 and Env3 and 10.5% across Env2 and Env4). There was no correlation across populations between the percentage of multifoliolate leaves and the leaf-to-stem ratio (data not shown).

Under moisture-favourable conditions, the three populations selected for quality-driven morphology had generally higher leaf-to-stem ratios than the conventional cultivar Prosementi across harvests, with significant differences ($p < .05$) for MSI030 and SI-Sel (Table 4). Cumulative DM yield over eight harvests under moisture-favourable conditions was highest for MSI030 and lowest for Miranda, with SI-Sel and Prosementi intermediate (Table 4).

The four environments differed ($p < .05$) for CP concentration, NDF concentration and NDF digestibility in leaves, stems and whole plants (Table 5). Results for ADF are not presented, given the extremely high correlation between ADF and NDF values ($r = 0.96$, $p < .05$) across the four environments and the very similar results of statistical analyses for these two traits. The ADL concentration is also not shown, as populations or environments did not differ for this character.

Crude protein content of leaves was highest in the moisture-favourable Env1 and lowest in the drought-stressed Env4 (Table 6).

TABLE 4 Mean and standard error (SE) values of average leaf-to-stem ratio (based on dry weight) and total dry-matter yield of four lucerne populations grown in moisture-favourable managed environments (Env1 and Env3 in Table 1)

Population	Leaf-to-stem ratio[a]	Dry-matter yield[b] (t ha^{-1})
MSI030	1.27 a	9.69 a
Miranda	1.20 ab	8.79 b
SI-Sel	1.27 a	9.04 ab
Prosementi	1.15 b	9.28 ab
$SE_{(df=5)}$	0.03	0.24
$LSD_{(p < .05)}$	0.09	0.73

[a]Average of six harvests: 2nd, 3rd, 4th in Env1; 1st, 2nd, 5th in Env3.
[b]Sum of eight harvests: 1st to 4th in both Env1 and Env3.

In environment pairs with same sowing date, fully irrigated moisture-favourable environments (Env1 and Env3) had higher leaf protein than drought-stressed environments (Env2 and Env4). Drought-stressed environments, however, tended to have higher stem protein concentrations than in moisture-favourable environments (Table 6).

TABLE 5 Summary of analyses of variance for the main forage quality traits recorded on leaves, stems and whole plants in the second harvest (early summer: 10 July 2012 for Env1 and Env2; 20 June 2013 for Env3 and Env4) from four lucerne populations grown in four managed environments (see Tables 1 and 3 for environment and population descriptions respectively)

	Probability of *F* test significance								
	Crude protein			Neutral-detergent fibre (NDF)			NDF digestibility at 24 hr		
Source of variation	Leaves	Stems	Whole	Leaves	Stems	Whole	Leaves	Stems	Whole
Environment (E)	$p < .01$	$p < .05$	$p < .01$	$p < .01$	$p < .01$	$p < .01$	$p < .05$	$p < .05$	$p < .05$
Population (P)	NS	$p < .05$	NS	NS	$p < .01$	NS	NS	NS	NS
E × P	NS	NS	NS	NS	$p < .05$	NS	NS	NS	NS

NS, not significant.

As a result, differences between stress levels were less clear-cut for whole-plant protein content. Env3 and Env4 did not differ for protein, but Env1 did have higher protein than Env2 (Table 6).

The NDF concentration in leaves, stems and whole plants was highest in the top-yielding environment Env3 (Table 6). The environment ranking for stem NDF concentration paralleled that for stem length (Tables 2 and 6). Along with the highest NDF content, Env3 also tended to have higher NDF digestibility than other environments, particularly compared to Env2, which had the lowest whole herbage digestibility (Table 6). Although not always supported by statistical significance, NDF digestibility tended to be higher in the moisture-favourable compared to the drought-stressed environment for both sowing periods (Table 6).

Only stem concentration of crude protein and NDF differed between populations across environments (Table 5). Stem protein content was highest in SI-Sel, intermediate in MSI030 and lowest in Prosementi and Miranda (Table 7). The short-internode population SI-Sel also had lower stem NDF concentration than any other population (Table 7). Population NDF positively correlated with the stem length ($r = 0.94$, $p < .07$) and negatively with the leaf-to-stem ratio ($r = -0.90$, $p < .10$).

4 | DISCUSSION

Harvesting by a growth-stage criterion is widely recognized as a reliable indicator of lucerne feeding value, owing to the influence of the phenological stage on forage yield and quality (Marten et al., 1988). The early flowering stage can provide an acceptable balance between good yield and high quality in lucerne forage (Sheaffer, 1983). Moreover, harvesting at the same phenological stage enables comparing lucerne germplasms for genetic differences in forage quality (Julier & Huyghe, 1997; Lenssen et al., 1991), avoiding confounding maturity with intrinsic quality.

Several studies have reported greater decrease in forage quality in lucerne stems than in leaves with advanced phenology (Marten et al., 1988). Although differences in quality can be found between basal and apical portions of the stem throughout the growing season (Christian, Jones, & Freer, 1970), the quality of the stem as a whole organ is a matter of concern in the improvement of feeding value (Julier & Huyghe, 1997; Lenssen et al., 1991), given its large proportion by weight in lucerne hay. Recent research has also shown that leaves and stems differ in crude protein fractions, with stems showing higher concentrations of non-protein and indigestible nitrogen, and lower content of true protein fraction than leaves (Hakl, Fuksa, Konečná, & Šantrůček, 2016). A specific selection for stem quality was supported by a recent genomewide association study, which suggested substantially different genetic control of forage quality traits in lucerne stems and leaves (Biazzi et al., unpublished data).

This study confirmed earlier observations on the close relationship between environment DM yield and stem height in lucerne (Pembleton, Donaghy, Volenec, Smith, & Rawnsley, 2010; Volenec, Cherney, & Johnson, 1987) and the reduction in stem length and number of internodes caused by drought stress (Afsharmanesh, 2009; Sheaffer et al., 1998). The moisture-favourable Env3 exhibited the top values for stem length and forage yield. However, that was

TABLE 6 Mean and standard error (SE) values of lucerne forage quality traits recorded on leaves, stems and whole plants in the second harvest (early summer: 10 July 2012 for Env1 and Env2; 20 June 2013 for Env3 and Env4) from four managed environments (see Table 1 for environment description)

	Crude protein (%)			Neutral-detergent fibre (NDF) (%)			NDF digestibility at 24 hr (%)		
Environment	Leaves	Stems	Whole	Leaves	Stems	Whole	Leaves	Stems	Whole
Env1	29.3 a	11.8 bc	20.5 a	23.2 b	61.5 b	42.5 b	43.7 ab	31.0 bc	37.3 a
Env2	26.7 b	12.3 ab	19.7 b	21.9 b	56.6 c	38.8 c	38.2 b	28.3 c	33.4 b
Env3	26.5 b	11.4 c	17.9 c	25.1 a	66.3 a	48.4 a	44.9 a	35.7 a	39.7 a
Env4	23.7 c	12.8 a	18.1 c	23.1 b	57.7 c	40.9 b	41.7 ab	34.1 ab	37.7 a
$SE_{(df=7)}$	0.4	0.3	0.2	0.4	0.4	0.6	1.8	1.4	1.1
$LSD_{(p < .05)}$	1.1	0.8	0.6	1.3	1.1	1.8	5.4	4.3	3.4

In each column, mean values followed by different letters were different at $p < .05$ according to the respective least significant difference (LSD).

TABLE 7 Mean and standard error (SE) values of stem crude protein and neutral-detergent fibre of four lucerne populations, recorded in the second harvest (early summer: 10 July 2012 for Env1 and Env2; 20 June 2013 for Env3 and Env4) over four managed environments (see Table 3 for population description)

Population	Crude protein (%)	Neutral-detergent fibre (NDF) (%)
MSI030	12.1 ab	61.1 ab
Miranda	11.6 b	60.2 b
SI-Sel	12.9 a	58.7 c
Prosementi	11.7 b	62.0 a
$SE_{(df=7)}$	0.3	0.4
$LSD_{(p < .05)}$	0.8	1.1

In each column, mean values followed by different letters were different at $p < .05$ according to the respective least significant difference (LSD).

also associated with the lowest leaf-to-stem ratio and highest NDF concentration in the whole plant and stems, indicating poor forage quality. A negative correlation between DM yield and leaf-to-stem ratio was observed by Ray, Townsend, and Muncy (1999a) and Ray, Townsend, Muncy, and Henning (1999b) in lucerne germplasm under both irrigated and drought-stressed conditions (up to $r = -0.75$, $p < .01$ under irrigation). Van Soest (1996) pointed out that drought conditions, if not too extreme, tend to provide high-quality forage because of increased leaf-to-stem ratio from shorter but leafier plants compared to moisture-favourable conditions. Likewise, Fonseca, Viands, Hansen, and Pell (1999) found a positive correlation between plant vigour and NDF, taller stems being intrinsically more fibrous than shorter ones. The result of Fonseca et al. (1999) was consistent with our finding that mean NDF stem concentration was lower in each drought-stressed environment than in each moisture-favourable environment at the same sowing date. Sulc et al. (1997) developed predictive equations including stem height as the main morphological variable for estimating lucerne NDF and ADF across a range of environments.

The optimum herbage growth of lucerne plants is established with temperatures around 27°C (McKenzie, Paquin, & Duke, 1988). Even under moisture-favourable conditions, high temperatures can lead to higher vapour pressure gradients from leaves to air often resulting in transient water deficit and reduced stomatal conductance that limit transpiration. The reductions in stomatal conductance also reduce photosynthesis and often reduce growth. Harvests 1–4 in Env1 and harvests 2–4 in Env3 were carried out during summer months, with maximum daily temperatures exceeding 30°C. The irrigation applied to these environments substantially re-integrated the PET during the cropping period and the limited total DM yield recorded over eight harvests could be accounted for, therefore, by the possible occurrence of a heat stress.

We observed lower CP in lucerne leaves and a higher concentration in stems under drought compared to moisture-favourable conditions, resulting in modest or no differences between irrigation treatments for whole-plant protein. This dynamic agrees with findings by Halim et al. (1990) under controlled irrigation levels. Their study, and that by Carter and Sheaffer (1983), reported no effect of water stress level on total herbage protein concentration. In contrast, Petit et al. (1992) reported higher herbage protein content with increasing drought stress. Based on an analysis of the dynamics of nitrogen content as a function of the measured aerial biomass, Lemaire et al. (1989) suggested that contradictory results of drought stress on nitrogen (and, hence, crude protein) concentration could partly be explained by two concomitant, contrasting effects, namely a direct negative effect of the stress on symbiotic nitrogen fixation and an indirect positive effect of stress through increased leaf-to-stem ratio (the protein concentration being much higher in leaves than in stems).

The observed high correlation between NDF and ADF concentrations confirmed by extensive literature (Guines et al., 2002; Hill & Barnes, 1977; Sheaffer et al., 1998), led Sheaffer et al. (1998) to suggest that either parameter provides an estimate of differences in forage quality. Lower NDF values in individual plant parts and in total herbage under stress confirmed earlier NDF results for total herbage (Deetz et al., 1996; Halim et al., 1990; Undersander, Cole, & Naylor, 1987). Lower fibre concentration (as ADF) with increasing drought stress was also reported by Petit et al. (1992). As suggested by Halim et al. (1990), drawing also from previous work, part of the fixed carbon may be used by plants under drought stress to synthesize compounds that enhance the cell osmotic adjustment, at the expense of cell wall development. The current lack of variation in ADL concentration across environments agreed with Deetz et al. (1996) while contrasting with Petit et al. (1992), who found ADL to decrease with increasing drought stress.

High-yielding, moisture-favourable environments displayed higher NDF concentrations along with higher NDF digestibility. Mertens (2009) asserted that, although a negative relationship between NDF content and NDF digestibility is expected in forages, decoupling this relationship might be possible.

The expression of the multifoliolate leaf trait was low and rather unpredictable across environments. It tended to be negatively affected by drought, but information on how environment affects multifoliolate leaf expression is scant. Under growth-chamber conditions, Juan, Sheaffer, and Barnes (1993a) reported that photoperiod had greater influence than air temperature on the development of multifoliolate leaves. However, they imposed fixed short- or long-day duration (roughly corresponding to the day length experienced in early spring and early summer, respectively, at our latitude) that could not be compared to the conditions of varying day length encompassed in our experiments.

The selection for a 'non-conventional' morphology contributed to increased leaf-to-stem ratio across the two moisture-favourable environments in the populations MSI030 (which encompassed high leaf-to-stem ratio in its selection history) and SI-Sel (selected for shorter internodes), both exceeding the conventional variety Prosementi for this trait. The population Miranda displayed intermediate values of leaf-to-stem ratio, along with limited expression of its multifoliolate trait, which could be rated between very low and low according to the expression classes defined by Juan et al. (1993a).

Indeed, only high expression levels of this trait are expected to significantly enhance lucerne forage quality (Juan, Sheaffer, Barnes, Swanson, & Halgerson, 1993b; Volenec & Cherney, 1990). Miranda's lower number of internodes per stem and shorter stems also accounted for the slight advantage over Prosementi in terms of leaf-to-stem ratio. The breeding strategy underlying the selection of MSI030 proved valuable, as it allowed combining top values of both leaf-to-stem ratio and forage yield. When assessed only at the second harvest, however, the population differences for leaf-to-stem ratio were not significant statistically and failed to reveal differences in quality features attributable to alternative morphology. Nonetheless, the population SI-Sel emerged for high stem quality, as revealed by its high value of stem protein concentration and low stem NDF content. Short-internode selections (from which population SI-Sel derived) already had lower stem fibre concentration than conventional germplasm in previous studies (Scotti et al., 2007). Higher stem quality of SI-Sel did not result in significantly higher forage quality on a whole-plant basis. Interestingly, the forage yielding ability of SI-Sel was not much affected by the depressing effect of shorter internodes on stem height.

Overall, this study suggested that environmental effects might have greater impact on forage quality than the genetic effects studied, even for a population set that partly included material specifically selected for quality-driven morphology.

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