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Characterization of cocksfoot (*Dactylis glomerata* L.) population for growth traits and summer dormancy

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Abstract

Cocksfoot is one of the four most important forages in the world, it is persistent and summer drought tolerant. Under Mediterranean climate, one way to produce without irrigation is to use plants resistant to drought, such as some perennial grasses as cocksfoot. The present work was conducted during 2011/2012 cropping season to evaluate some traits of progeny generated from a cross between a summer dormant genotype Kasbah cv and a summer active genotype from Medly cv under Mediterranean climate.The aim of this study was to gather hybrids pools according to common features with relation to summer dormancy. Measurements of dry matter yield, heading date, plant height, plant growth rate, senescence and summer dormancy were taken for the 229 F1 hybrids. Results showed a large variability between hybrids for all traits. 4% of hybrids were more dormant than the parent Kasbah, 35 % of hybrids yielded more than Medly, 73.8 % of progeny exceeded parents growth rate, 60 % had the greatest biovolume, more important than both parents. Significant correlations (P <0.0001) were found between all traits except heading date and senescence. Cluster analysis (CA) revealed eight groups and subgroups which showed 80 % of phenotypical similarities within hybrids and parents.

1. Introduction

Morocco and the whole Mediterranean region are one of the so-called climate change hotspots. Climate changes are considered as a major obstacle to agricultural production, including perennial grasses [1]. Perennial grasslands are expected to produce over many years and their sustainability is associated with yield stability and long-term resilience [2]. For forage plants growing in areas affected by prolonged and severe summer drought, the most important agronomic characteristic is not the ability to produce during drought, but the ability to survive summer, recover in autumn and grow actively during the cooler rainy seasons [3]. Indeed, the persistence of perennial herbaceous plants is mainly determined by plant survival over successive summer droughts [4].

Dactylis glomerata L. (Orchardgrass or Cocksfoot) is the fourth most important forage grass in the world [5], classified as one of the most important perennial grasses, specifically in arid conditions [6]. Cocksfoot is widely distributed in most European countries, North America such as the USA and Canada, South America, Australia, New Zealand, and Asia [7]. It includes many subspecies, with diploid (2x), tetraploid (4x) and hexaploid (6x) cytotype while the tetraploids display wide ecological tolerance [8]. The Mediterranean cocksfoot populations have developed valuable adaptive traits such as summer dormancy as an adaptive response, determined as an absence of growth in summer despite irrigation [9]. Defined as one of the most important traits that grasses developed in harsh environments known as "an endogenously controlled and coupled series of processes comprising the cessation of leaf growth and senescence of herbage expressed exclusively in summer even under non-limiting moisture" [9]. Summer dormancy confers superior persistence under drought and can be useful to achieve a range of agronomic and environmental goals [10]. It is associated with reduced water consumption

[11] and to increased survival [12]. This strategy can only reliably be identified and measured in plants that are not experiencing drought, i.e. when they are well watered [13].
Under dry conditions, summer dormancy appeared when drought or reduced water availability is the main factor limiting crop production [14]. It is easily confused with the drought resistance response 'dehydration avoidance' [9]. However, we can distinguish these two responses when water deficit is relieved [13]. Two categories of summer dormancy were defined according to Volaire and Norton [10]. The first one is an incomplete dormancy when plant produced less with moderate levels of foliage senescence [10], as in *tall fescue* [15] and *phalaris* [16]. The second type is a complete dormancy, observed when population ceased growth completely during the summer a minimum of four weeks. It induced dehydration of leaf bases. Nevertheless, complete summer dormancy seems be associated with low productivity in cocksfoot [9]. The Mediterranean cultivar of cocksfoot subsp. *hispanica* from Morocco is characterized by complete summer dormancy [17].
The main objective of this study was to gather hybrids pools according to common features with relation to summer dormancy. In addition, this work helps to select promising genotype that can be used for the further breeding program.

2. Materials and methods

2.1. *Plant material and growth conditions*
Two cultivars of cocksfoot and their hybrids were compared under field conditions. The cultivar 'Kasbah' *Dactylis glomerata ssp hispanica,* originated from Morocco and bred in Australia exhibits complete summer dormancy, drought resistance [12]. 'Medly' cultivar *Dactylis glomerata ssp glomerata* is an early flowering variety, summer-active of Mediterranean origin [19, 20]. Medly is more productive than Kasbah but less tolerant to severe drought [21, 22]. Parents and hybrids were all tetraploid (2n=4x=28).

Experimental site characterization and conditions
The field experiment was conducted at the Guich experimental station of INRA, Rabat/Morocco (Latitude 34°03' N, Longitude 06°46' W, Elevation 10, 5 masl) on a sandy soil, pH 7. Hybrids were generated from an intra-specific crossing between two genotypes of cocksfoot: a genotype of the summer dormant cultivar Kasbah from Moroccan origin and a genotype of the summer active Mediterranean cultivar Medly. 283 hybrids and parents were cloned, replicated and were transplanted after two months in a nursery at the Guich experimental station of INRA in February 2011.The trial was designed in a randomized block with tree replications. Plants were 1 meter spaced from both sides. The field was fertilized before planting with 28, 56, 28 kg/ha of N, P, K respectively. Plants were harvested on four dates, hand cuts were made at 10 cm height: the 31st of January, the 2nd of April, the 15th of May and the 11th of September. At tillering stage, a nitrogen fertilizer (33 % ammonium nitrate) was applied at a rate of 40 kg of nitrogen /ha after the first three cuts. The trial was irrigated during summer, twice a week to evaluate summer dormancy. Hybrids characterization was conducted over 2011 and 2012, only 2012 data are presented in this paper. All measurements were made on 229 genotypes.

Climatic conditions during the experiment
Monthly rainfall and temperatures are shown in figure 1.

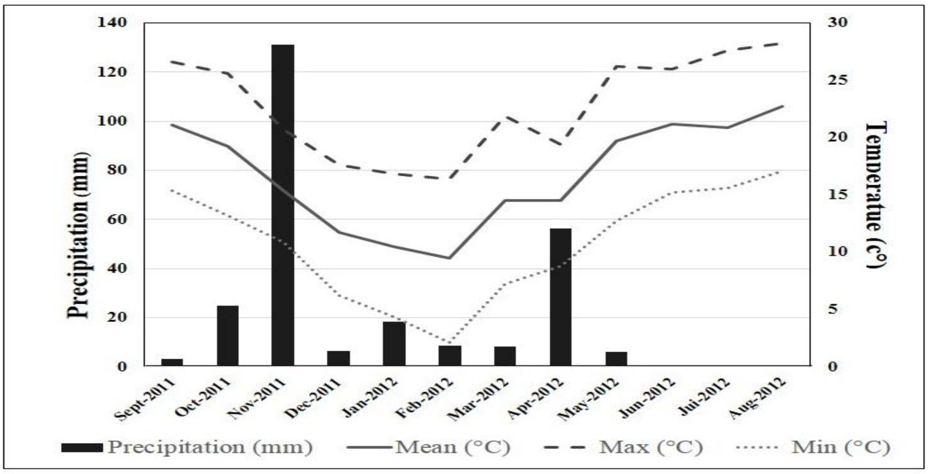

Figure 1: Distribution of monthly rainfall (mm), temperature (min, max, mean) in °C recorded for 2011/2012 at the Guich/Rabat station.

Cumulative rainfall reached 263 mm in 2012. This value was quite low than the normal average of this region (420 mm). The average for rainy months in 2012 was 26.2, 26, 27.6 and 28.2 mm respectively for April, May, June, July, and August.

2.2. Plant measurements
The following traits were recorded in the field: Dry matter yield (g/plant) of individual plants, heading date (days), plant height (cm). These traits were measured as mentioned in our previous publication [18]. Plant growth rate (PGR) was estimated by measuring plant height weekly during 6 weeks from 9 February to 8 March and reporting to time in degree-days (°Cd).
The biovolume was calculated for each genotype using the plant height (cm) and the plant diameter (cm), measured respectively on 8 March and 2 April before the second cut. The senescence of aerial tissues in midsummer and summer dormancy were recorded according to Norton et al. [9, 13] index. In an attempt to describe the hybrids, a multi-trait characterization was carried out; a hierarchical clustering method was elaborated using data from fields in Rabat.

2.3. Statistical analysis
Statistical analysis was performed using the SAS (Statistical Analysis System, software) for 229 hybrids. The data were subjected to analysis of variance (ANOVA). Pearson correlations were performed between studied traits and mentioned in (Table). In case of significant difference between lines, cluster analysis (CA) was carried out to determine the various groups of parents and hybrids. P value was highly significant ($P < 0.0001$) for all measured traits.

3. Results and discussion
3.1. Dry matter yield
During the establishment year, total biomass productivity was ranged from 3.5 to 202 g/plant. Figure 2 shows different classes of genotype for total dry matter in 2012. The parent varieties Kasbah and Medly produced respectively 57.2 and 91.0 g/plant with the population average of 93 g/plant. 35 % of hybrids yielded more biomass than the productive parent Medly, while 27 % of hybrids had intermediate dry matter yields between both parents. Four harvests were performed, in winter, spring and summer. Significant differences were found for dry matter yield accumulated in winter cut ($P < 0.0001$), dry matter yield mean was 10.2 g/plant quite less than the average reached at the second cut (46.8 g/plant).

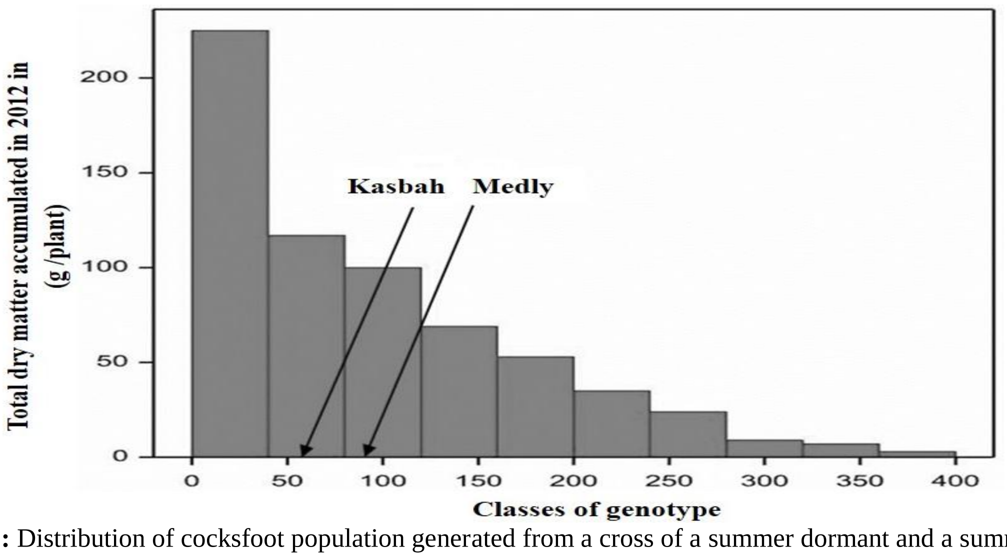

Figure 2: Distribution of cocksfoot population generated from a cross of a summer dormant and a summer-active cultivars in classes for total dry matter accumulated in 2012 in (g /plant) with parents, Medly and Kasbah.

Spring biomass differed significantly ($P < 0.0001$) among genotypes and showed a high yield with a large variability. Dry matter yield mean was 76.6 g/plant. A positive correlation ($r = 0.70$, $P < 0.0001$) was found between spring biomass and plant height, which are two parameters determining plant production. A similar correlation was reported by Metfi et al. [23] and Volaire [24] on perennial grasses. In summer, genotypes produced less biomass compared to other harvests because a majority of genotype ceased production during this period.

3.2. Plant growth rate (PGR) and biovolume

Plant growth rate and biovolume were measured in spring. The biovolume showed the vigor of each genotype. The analysis of PGR showed significant differences between genotypes and parents ($P < 0.0001$). The population average was 0.57, the parent Kasbah had a rate 0.40 lower than that for the productive parent Medly, which achieved 0.46 ($mm°C^{-1}d^{-1}$). About 73.8 % of our population had a superior rate, higher than those for parents. PGR was positively correlated to spring biomass and annual forage yield, respectively ($r = 0.63, 0.76, P < 0.0001$), and which are two parameters determining plant productivity, especially plant vigor. Whereas PGR was negatively correlated to senescence ($r= - 0.15, P < 0.0001$). Similar correlation was found between PGR and summer dormancy ($r = - 0.28, P < 0.0001$) as also found by Shaimi et al. [14].

Significant differences ($P < 0.0001$) appeared between genotypes for plant biovolume. The population average was 2164 cm^3, Medly achieved 1712 cm^3 while Kasbah was smaller with 1262 cm^3. 60 % of hybrids had the greatest biovolume than parents, thus had an important vigor than other genotypes (figure 3).

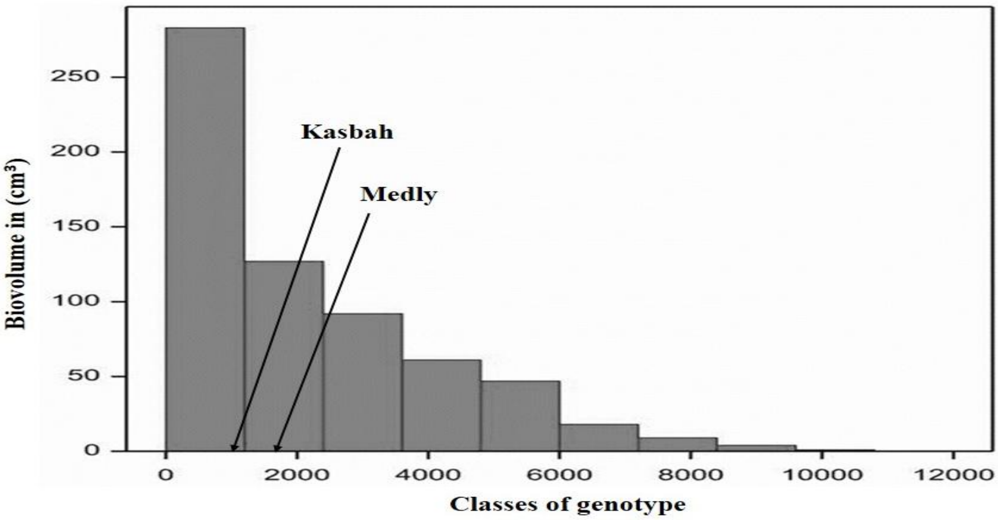

Figure 3: Distribution of cocksfoot population generated from a cross of a summer dormant and a summer-active cultivars in classes for biovolume noted in 2012 in (cm^3) with parents, Medly and Kasbah.

3.3. Plant Height

Plant height at heading for all genotypes ranged from 28 to 80.6 cm, with a population height average of 56.9 cm. The parent Kasbah was shorter than Medly. Significant differences were found ($P < 0.0001$) between all genotypes. 66.8 % of genotypes were taller than both parents, while 22.5 % were shorter than the parent Kasbah. Plant height was inversely correlated to senescence ($r= - 0.16, P < 0.0001$) and to heading date ($r= - 0.59, P < 0.0001$) with a large variability among genotypes. A similar correlation was reported in ryegrass populations [25].

3.4. Heading date

Significant differences appeared between genotypes ($P < 0.0001$) for heading date which ranged from 55.0 to 99.3 days from first of January 2012 (figure 4), while it ranged from 83 to 125 days in cocksfoot population from a Mediterranean origin [20]. The average heading date was 76.5 days. The parent Medly was earliest than Kasbah, this is consistent with earlier findings [14, 19]. Heading date was negatively correlated to spring biomass and total dry matter, respectively ($r= - 0.54, r= - 0.52, P < 0.0001$), a similar correlation was noted in cocksfoot population [26, 27].

3.5. Senescence of aerial tissues

Senescence score varied from 8.3 to 95.0 % within the population. The highest score was achieved by genotype 91 not significantly different than the parent Kasbah. The non-dormant parent Medly has less senescence score of 38.3 %. 62.4 % of the population had a senescence included between parents score. 4.0 % had highest senescence scores than Kasbah, which is completely dormant under summer irrigation [17, 15], these genotypes are expected to re-growth after summer drought and show superior survival. Under summer irrigation, negative correlation was found between herbage senescence and spring biomass also annual biomass productivity, respectively ($r= - 0.22, r= - 0.25, P < 0.0001$).Whereas, these correlations were not tight and highly senescent and productive genotypes were noted between offspring.

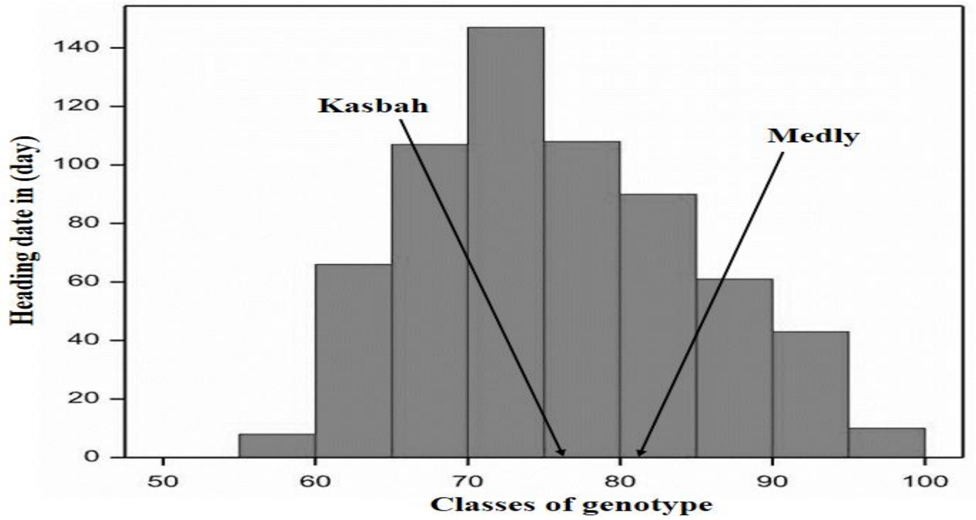

Figure 4: Distribution of cocksfoot population generated from a cross of a summer dormant and a summer-active cultivars in classes for heading date noted from the first of January 2012 in (day) with parents, Medly and Kasbah

3.6. Summer dormancy index (SDI)

Results showed significant differences (P < 0.0001) between genotypes for the summer dormancy index (SDI), varied between 4.1 and 9.8 with a large variability between genotypes. The average was 8.9.The dormant parent 'Kasbah' has a high dormancy index of 9.7. It was reported that higher dormancy levels, generally but not always, promote survival in drier, hotter areas at lower altitudes [28, 29, 30, 16]. Other research reported that Dormancy Index ranged from 0.1 to 9.4 in *Phalaris* [31]. A negative correlation was established between heading date and summer dormancy (r = − 0.26, P < 0.0001) while a weak positive correlation (r = 0.002, P < 0.0001) was found between those parameters in an earlier study on cocksfoot population [20]. On the other hand, summer dormancy was positively correlated to leaf senescence (r= 0.24, P < 0.0001), Norton et al. [15] suggested that leaf senescence could be used to identify summer dormant plants in his study on *Festuca arundinacea Schreb*. Summer dormancy seems to be positively correlated to spring biomass and to total biomass, respectively (r = 0.28, r = 0.23, P < 0.0001), whereas dormancy has been shown to be negatively correlated to annual forage production [14].

Table: Pearson's correlation coefficients between measured traits in cocksfoot's population

Measured traits	PH	TDM	SB	SDI	Senescence
HD	– 0.59	– 0.52	– 0.54	– 0.26	0.06 (NS)
PGR		0.76	0.63	− 0.28	– 0.15
PH			0.70	0.35	– 0.16
SB				0.28	– 0.22
SDI				1.00	0.24
TDM				0.23	– 0.25

HD: heading date; **PH:** plant height; **PGR:** plant growth rate; **SB:** spring biomass; **SDI:** summer dormancy index; **TDM:** total dry matter yield accumulated in 2012.
All correlation are very significant at (P < 0.0001)
NS: not significant

3.7. Multivariate analysis of several traits

In this study, a hierarchical clustering method was used in data from fields in Rabat. A large number of genotypes made interpretation quite difficult after using Duncan grouping of hybrids population. Cluster Analysis (CA) was used to graphically classify genotypes and parents according to measured traits. Figure 5 presents the corresponding dendrogram using plant height, heading date, spring dry matter and senescence score. Cluster analysis highlighted two major groups (A and B) with different numbers of genotypes. Taking (0.8) as a level of similarity, each group was subdivided into three subgroups with a different number of genotypes. Indeed, eight groups and subgroups were defined mainly. Two major groups were defined with a similar number of genotype in each one.

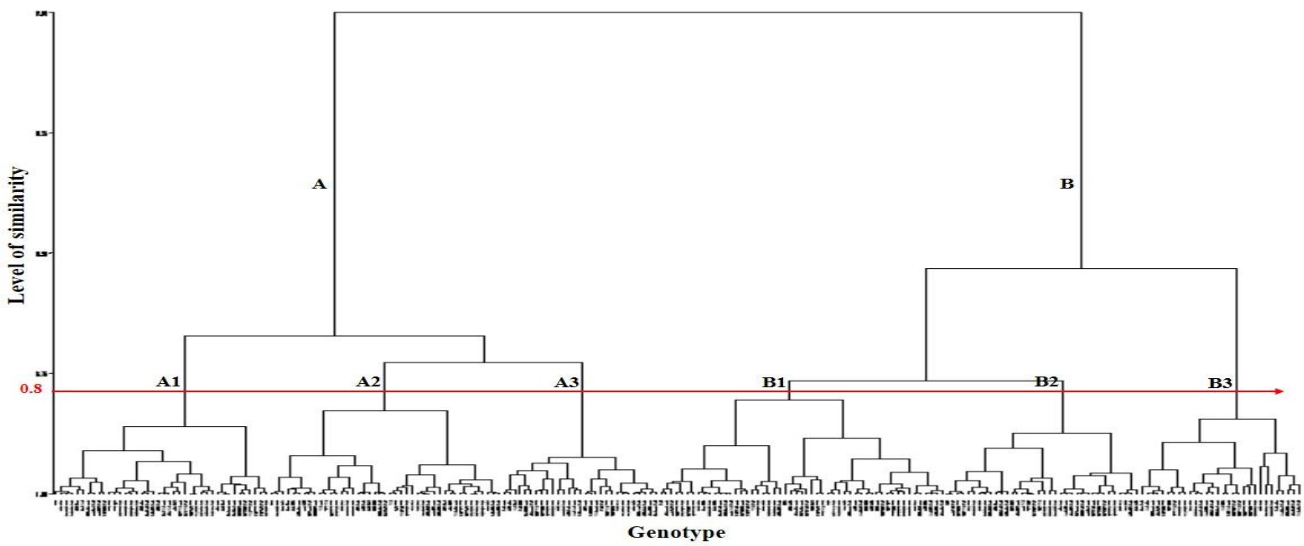

Figure 5: Cluster dendrogram showing the distribution of our population into eight groups and subgroups, using Duncan grouping based on four traits: Plant height, Heading date, Spring biomass and Senescence in the irrigated experiment at the Guich /Rabat station.

Group A was selected according to its high senescence scores. It contains 112 genotypes including the parent Kasbah, which represents 48.5 % of the population.Three subgroups are distinguished, the first **subgroup A1** contained 40 genotypes, which produced between 34.5 and 80.8 g/plant, 87 % of this subgroup had a senescence scores from 20 to 60 %. **Subgroup A2** included 43 genotypes, contained the summer dormant parent Kasbah. These genotypes produced less dry matter than A1 with a level of senescence up to 64 %. 80 % of this group produced between 20.0 and 70.3 g/plant and had the highest score of senescence. All these genotypes were shorter than 62 cm, furthermore reached the heading stage at 88 days, thus earliness similar to Medly. **Subgroup A3** included 29 hybrids, with senescence score less than 63 % and accumulated less than 39.3 g/plant as spring biomass. These genotypes reached heading stage earlier than genotype of A2.

Group B was selected according to his high spring dry matter yield. It contains 119 genotypes including Medly which represents 51.51 % of the population. Three subgroups are distinguished. **Subgroup B1** included 53 genotypes, accumulated a good level of spring dry matter from 64.6 to 141.4 g/plant. These genotypes had a senescence score from 33.3 to 93.3 %. **Subgroup B2** comprised 36 hybrids, among them the productive parent Medly. The level of spring productivity was less important than that accumulated by genotypes from subgroup B3. Senescence of aerial tissues was ranged from 11.6 to 47.5 %. **Subgroup B3** represented by 30 hybrids, which accumulated a good level of dry matter yield, exceeding 119 g/plant, more productive than the productive parent Medly. This subgroup is characterized by greater height (up to 58 cm), more important than both parents. These genotypes had a score of senescence less than 66 %.
We conclude that the present cocksfoot population can be divided to two groups according to parent characteristics. The first group with a high level of senescence in mid-summer while the second one with an important level of productivity, especially in spring.

Conclusion

Characterization of hybrids issued from a crossing between two contrasting varieties of cocksfoot (*Dactylis glomerata* L.) showed a considerable variability for plant height, heading date, dry matter yield, plant growth rate, senescence score, and summer dormancy index. Several correlations were found between studied parameters and yields of the hybrids appeared quite promising. Some hybrids had a good level of spring biomass than parents, and other genotypes had a better senescence score than parents. The cluster analysis (CA) identified different groups with phenotypical similarity which confirmed the variability between hybrids and parent for different measured traits. We found, in progeny, some genotypes more productive and more dormant than both parents which are widely adapted to Moroccan field conditions. Especially, they constitute a promising genotype that can be used for the further breeding program. These results are encouraging and suggest that it’s possible to create cocksfoot varieties both productive and summer dormant.

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