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Promising forage resources for production systems in Mediterranean areas

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Abstract. Extensive livestock production is a basic socio-economic feature of Mediterranean agriculture threatened by overgrazing of natural grasslands and by climate change. Stress-tolerant forage resources are increasingly needed, therefore, to enhance the sustainability of Mediterranean crop-livestock systems. Assessing the response of the available germplasm of a few, key species across different stress levels of the Mediterranean area can provide information on best plant types, genetic resources and adaptation strategies. A large set of experiments (here summarised) was performed for over a decade on both sides of the western Mediterranean basin, indicating that germplasm adapted to dryland conditions can be found in all forage species. This germplasm could be directly used for cropping or as parental material in breeding programmes. A clear genotype × environment interaction was generally found for yield, emphasising the need of selecting specific germplasm for different environments. Legume-based crops displayed higher forage quality and farmers' appreciation than pure-stand crops of cereals or grasses.

Keywords. Adaptation – Drought tolerance – Genotype × environment interaction – Selection – Summer dormancy.

Ressources fourragères pour les systèmes de production des zones méditerranéennes

Résumé. *L'élevage extensif est une caractéristique socio-économique de base de l'agriculture méditerranéenne, menacée par le surpâturage des prairies naturelles et par le changement climatique. Des ressources fourragères tolérantes au stress sont donc de plus en plus nécessaires pour renforcer la durabilité ces systèmes d'élevage. L'évaluation de la réponse du matériel génétique disponible de quelques espèces clés à différents niveaux de stress de la région méditerranéenne peut fournir des informations sur les meilleurs types de plantes, ressources génétiques et stratégies d'adaptation. Un grand nombre d'expériences (résumées ici) a été réalisée pendant plus d'une décennie des deux côtés du bassin méditerranéen occidental, indiquant que du matériel génétique adapté aux conditions de zones sèches peut être trouvé dans toutes les espèces fourragères. Ce matériel génétique pourrait être directement utilisé pour la culture ou comme matériel parental dans les programmes de sélection. Une interaction génotype × environnement a généralement été trouvée pour le rendement, soulignant la nécessité de sélectionner des variétés spécifiques pour différents environnements. Les cultures à base de légumineuses ont montré une meilleure qualité de fourrage et une plus grande satisfaction des agriculteurs que les monocultures de céréales ou de graminées.*

Mots-clés. *Adaptation – Tolérance à la sécheresse – Interaction génotype × environnement – Sélection – Dormance estivale*.

I – Introduction

Crop-livestock systems have an outstanding importance in the Mediterranean basin, safeguarding local economies, protecting the agricultural environment and the rural landscape, fulfilling the steadily growing demand of animal products, and representing an economic buffer for smallholders. The productivity of these systems will be affected by climate change, because of the predicted greater incidence of drought and higher frequency of extreme climatic events. The direct negative effects of climate can be exacerbated by overexploitation of forage resources and increasing costs and/or decreasing availability of irrigation water. Excessive exploitation resulted in decreased feed production and increased erosion and risk of desertification of natural grasslands. To reverse this trend, forage crops able to withstand climatically adverse conditions and provide more feed at sustainable costs are needed. In the drier Mediterranean regions, currently cultivated forages mainly include mixtures or monocultures of annual crops. Annual species display adaptation to severe spring-summer drought thanks to their growth largely concentrated in the cool season, when water is more available, and their ability to survive through the dry period as seeds. Compared to annuals, however, perennial species have quicker regrowth at the onset of autumn rains and better exploit the residual moisture in late spring, thus allowing to extend and regularise the feeding season, mostly concentrated in spring for the annuals. A need arises, therefore, of identifying perennial crops able to persist under Mediterranean climatic conditions. Perennials should possess intrinsic drought tolerance or any physiological mechanism enabling their survival under severe summer drought. Several forage species could be potentially useful for the Mediterranean region. However, as the funding for forage research and breeding in the area is limited at the national and international level (Porqueddu *et al*., 2016a), a careful identification of a few promising species on which concentrating joint efforts and resources is required. Nitrogen-fixing forage and feed legumes with improved tolerance to major abiotic stresses may play a crucial role in strategies of climate change adaptation and mitigation, while enhancing the economic and environmental sustainability of the Mediterranean agriculture. Modelling cultivar responses in multi-site evaluation trials can help appreciating and predicting differences of adaptive patterns across a range of increasingly stressful conditions. Two large international projects on forage crops were carried out involving institutions and researchers from both sides of the Mediterranean Sea. Their major goal was to boost the identification of new and better adapted germplasm across Mediterranean conditions and to offer innovative solutions in livestock feeding and sustainable farming in dryland agriculture. This contribution summarises over a decade of experiments performed in the western Mediterranean basin.

II – Lucerne

Lucerne (*Medicago sativa* L.) is the main forage crop in south-European countries and the main perennial forage species in North Africa. By its deep rooting system, it can tolerate periods of drought. However, in very dry conditions lucerne is only grown under irrigation. Reducing or withholding irrigation during the period of lowest water-use efficiency (summer months) might be a solution deserving verification, provided that suitable germplasm is identified. Soil salinity is a major stress affecting crop production in drought-prone regions of North Africa. A comprehensive study encompassed landrace and variety germplasm from North Africa, southern Europe, Australia and USA and evaluated dry matter (DM) yield over three years across 10 agricultural environments of Algeria, Tunisia, Morocco and Italy, four of which were rainfed, one was continuously irrigated (oasis management) and five were irrigated but with a nine-week irrigation withholding during summer (Annicchiarico *et al*., 2011a). The crop persistence was good in all environments, but the environment mean yield was closely related with the annual and spring-summer (April-September) water available. A yield reduction of about 40% with rainfed cropping was paralleled by a water saving of over 60% compared to the irrigation regime (with summer withholding). The latter had proportional yield reduction and water saving compared to the oasis management. The economic dis-

advantage of lower forage yield might be outweighed by the advantages provided by the saved water with alternative non-agriculture uses or with the irrigation of high-value orchard crops. These results suggested considerable scope for water savings in the Mediterranean basin through rainfed cropping or withheld summer irrigation, provided drought-tolerant germplasm is available.

Crossover genotype × environment (GE) interaction between top-yielding cultivars occurred across the environments. Spring-summer water available, total number of harvests and soil salinity were identified as significant environmental covariates in a factorial regression model explaining over half of the GE interaction variation (Annicchiarico *et al*., 2011a). The old Sardinian cultivar Mamuntanas, which evolved under rainfed cropping and moderate annual rainfall, displayed specific adaptation to severely drought-stressed environments, whereas landraces from North Africa, which evolved in irrigated environments, were generally not adapted to these environments. One variety (Ameristand 801S) and one Moroccan landrace (Erfoud 1) were specifically adapted to salt-stress environments. Apart from saline-soil conditions, the variety SARDI 10 proved to be outstandingly adapted to all conditions. SARDI 10 possibly being an exception, the extent and complexity of the GE interaction effects observed in our study discouraged the breeding of widely adapted varieties across the western Mediterranean basin. To improve our knowledge on the relevant adaptive traits, five cultivars with contrasting adaptive responses were further evaluated under managed environments to identify physiological and morphological traits associated with specific adaptation to drought-stressed and irrigated environments (Annicchiarico *et al*., 2013a). The results emphasised the difficulty of combining traits of environment-specific adaptive value into one widely adapted variety, thus supporting the selection of varieties specifically adapted to contrasting environments.

Based on the multi-site evaluation, a gene pool was established at CREA, Lodi, intercrossing the three outstanding cultivars Mamuntanas, Erfoud 1, and SARDI 10. This gene pool included 152 half-sib progenies and was meant as a common genetic base for phenotypic selection targeted to stress-prone conditions in the Mediterranean basin, with trials established in Lodi, Italy, under severe drought stress (using a rainout shelter), in Alger (Algeria) under rainfed conditions, in Marrakech (Morocco) under summer suspended irrigation, and in Médenine (south Tunisia) under continuous irrigation with saline water (EC_e: 9.73 dS/m). In each location the top 10% progenies were identified based both on cumulated DM yield over three growing seasons and final persistence, to be intercrossed and advanced in the selection process. The phenotypic advantage of the promising progenies over the whole set of material was outstanding in all sites for DM yield and sizeable for the final persistence, except in Marrakesh, where plant survival was sustained by the rather favourable moisture conditions (Table 1).

Table 1. Mean values of cumulated dry-matter yield (DMY) and final persistence of the top 10% lucerne progenies identified in each of four evaluation environments in comparison with the overall trial mean values

Germplasm group	Marrakesh		Alger		Médenine		Lodi (managed stress)	
	DMY (t/ha)	Final persist.	DMY (t/ha)	Final persist.	DMY (t/ha)	Final persist.	DMY (t/ha)	Final persist.
Top 10%	46.52	91.1	25.67	53.6	91.17	19.8	18.67	35.9
Trial mean	37.31	88.6	20.78	47.5	73.64	16.7	13.90	25.8
Difference	+24.7%	+2.8%	+23.5%	+12.8%	+23.8%	+18.6%	+34.3%	+39.1%

III – Cocksfoot

Lucerne can also be grown in mixed stand with a forage grass to improve the crop yield, quality and seasonal distribution, and the efficiency of nitrogen utilisation. Cocksfoot (*Dactylis glomerata* L.) and tall fescue (*Festuca arundinacea* Schreb.) are the most important perennial grass species of potential interest across the Mediterranean basin. Within the Mediterranean germplasm of cocksfoot, two contrasting strategies of adaptation to drought can be found. One strategy relies on summer dormancy, that is, a complete aerial senescence at the end of spring irrespective of the water availability (Volaire *et al*., 2009). This mechanism is mostly found in North-African germplasm of *D. glomerata* subsp. *hispanica* (Roth) Nyman and is especially useful for plant survival under severe stress. Incomplete dormancy can be found in Mediterranean populations of cocksfoot (Volaire *et al*., 2009). The other adaptation strategy is dehydration tolerance, by which enough moisture is maintained in leaf basal tissues even at low soil water potential (Volaire, 2008).

Our main study on cocksfoot involved three rainfed test sites in southern Europe (one each in Italy, France and Portugal) and three in North Africa (one in Morocco and two in Algeria) and encompassed diversified germplasm, of Mediterranean or continental type, and with complete, incomplete or absent summer dormancy (Annicchiarico *et al*., 2011b). The best predictive model for yield was a factorial regression as a function of the site (April-September) drought stress (estimated as long-term potential evapotranspiration minus the actual water available for the crop). The large genotype × location (GL) interaction observed for yield was mostly associated with the summer dormancy trait. Completely dormant germplasm was specifically adapted to the most severe stress, whereas the Mediterranean subsp. *glomerata* germplasm tended to be specifically adapted to moderate drought, although some variation was present in their response to drought stress levels. The continental germplasm was not adapted anywhere. The completely summer-dormant cultivar Kasbah had the highest final persistence across sites, highlighting an intrinsic advantage for this physiological trait in terms of general persistence across environments (possibly conferred by a reduced metabolic activity in summer), in addition to its specific-adaptation effect leading to relatively better yield under severe stress. Should long sward duration be required in severely drought-prone, extensive farming systems, the completely dormant germplasm of subsp. *hispanica* could be a useful plant type for introduction into cultivation or for breeding. When the growing conditions are less stressful, the Mediterranean germplasm of subsp. *glomerata* is a better option than the completely dormant material owing to its higher forage yield potential.

IV – Tall fescue

Two geographical types are recognised in tall fescue, of which the so-called Mediterranean type showed good persistence under summer drought stress (Pecetti *et al.*, 2007). Complete summer dormancy was not reported in the species, but an incomplete dormancy was observed in some Mediterranean tall fescues (Norton *et al*., 2008). Other Mediterranean germplasm relies on dehydration delay related with water uptake from deep roots (Lelièvre *et al*., 2011), although mechanisms of dehydration tolerance should not be overlooked (Norton *et al*., 2006).

We evaluated Mediterranean germplasm of tall fescue for DM yield over three years and final persistence under rainfed conditions across the six rainfed sites previously described for the cocksfoot study (Pecetti *et al*., 2011). The cultivar yield response across sites was best predicted by a factorial regression as a function of the site spring-summer (April-September) drought stress. The yield potential also proved to be restricted by cold winter temperatures. The incompletely-dormant varieties Flecha and Fraydo showed positive adaptation to sites with highest spring-summer drought stress. Their adaptation pattern was consistent with the higher level of drought stress of their selection environments compared to the other tested cultivars. Although crossover GL interaction was remarkable in this study,

Flecha tended towards wide adaptation across sites. This pattern suggested that selection of tall fescue germplasm with wide adaptation across diversified Mediterranean drought stress levels can be feasible, unlike in cocksfoot where specific-adaptation responses were remarkable and largely accounted for by the presence of the complete summer dormancy mechanism.

When directly compared, Mediterranean tall fescue consistently outyielded Mediterranean cocksfoot (Pecetti *et al*., 2009; Lelièvre *et al*., 2011; Annicchiarico *et al*., 2013b). This yield advantage of tall fescue may derive from higher water-use efficiency and greater growth across autumn and winter, as well as deeper and larger root system compared to cocksfoot (*Lelièvre et al*., 2011). In this sense, tall fescue appears to meet the desirable ideotype for a perennial species, which is provided with endurance to summer drought coupled with high growth rate when moisture availability is non limiting.

V – Pea

Pea (*Pisum sativum* L.) is the main feed grain legume along with faba bean (*Vicia faba* L.) in southern Europe, while it is mostly grown for forage in mixture with a cereal in the Maghreb. Pea has a remarkable flexibility of use, as it may be harvested at crop maturity for grain (used as a concentrate) and straw (used as a fodder, with nutritive value slightly lower than that of an average lucerne hay) or harvested earlier for hay production. If unfavourable climatic conditions led to poor grain yield, it may also be grazed at maturity. Elite plant material was identified and used to produce sets of recombinant inbred lines (RIL) for breeding purposes (Annicchiarico and Iannucci, 2008). Seed of three paired crosses between the promising varieties Attika, Isard and Kaspa was multiplied at CREA, Lodi, and made available for the phenotypic evaluation. For each *cross*, a set of about 100 RILs was evaluated in three experiments carried out in Lodi (Italy) under severe terminal drought (under a rainout shelter), Alger (Algeria) under rainfed conditions and moderate drought stress, and Marchouch (Morocco) under rainfed conditions and severe stress. Both in Marchouch and Alger, the aim was to identify the three best grain yielding lines within each cross, to be advanced to the next selection steps. The yield advantage (in phenotypic terms) of the preliminarily selected germplasm over the entire set of evaluated lines and the parent cultivars was outstanding (Table 2). The selection requirements for a pea variety in Italy entailed additional factors than just adaptation to severe terminal drought, although this is a situation often experienced in harsh Mediterranean areas of southern Italy. The recorded data highlighted the positive features of four lines, out of which one new variety adapted to Italian conditions was proposed for registration.

Table 2. Grain yield mean values of preliminarily selected pea lines (three lines within each cross: Attika x Isard; Kaspa x Attika; Kaspa x Isard) from the evaluation under rainfed conditions in Marchouch, Morocco, and Alger, Algeria, of 315 lines from three crosses, and grain yield mean values of the whole set of lines

Germplasm group	Marchouch, severe drought (56 mm rainfall)	Alger, moderate drought (348 mm rainfall)
	Grain yield (t/ha)	Grain yield (t/ha)
Selected lines	0.84	2.68
Trial mean	0.36	1.37
Difference	+133%	+95%

VI – Legume-based forage mixtures

We aimed at optimising the cultivation and use of pea-based and lucerne-based forage crops in different drought-prone Mediterranean environments, by assessing different pea-cereal and lucerne-grass mixtures, other mixtures with legume species, and pure-stand crops, in terms of forage yielding ability, resilience, forage quality and acceptability by farmers. Legume-based mixtures raise potential interest to increase crop forage yield and/or quality through resource complementarity of different species, but their actual performance and acceptability by farmers need to be verified. Key questions need to be solved to design and propose innovative forage crops, such as: Choosing annual or perennial crops? Growing legumes in pure stand or in mixture with cereals/grasses? Growing binary or more complex mixtures? Is there more suitable legume crop ideotypes (e.g., in terms of plant morphology) than others to grow in mixtures? A study was carried out across the drought-prone Mediterranean sites of Sassari, Sardinia, Marchouch, inland Morocco, and Sétif, inland Algeria. The experiment included 16 annual and 9 perennial forage crops, encompassing pure and mixed stands of annual legumes (pea, common vetch, Narbon vetch), cereals (oat and triticale), lucerne and grasses (cocksfoot and tall fescue) (Annicchiarico *et al*., 2017a; 2017b). A tall and a semi-dwarf pea line (the plant stature depending on the presence or absence of dwarfing genes) and an upright growing and a semi-erect lucerne population were evaluated as possible plant ideotype alternatives. All combinations of legume-cereal binary mixtures and two complex (4-components) mixtures (one with two pea types and two cereals; the other with two vetch species and two cereals) were evaluated for annual crops, while perennial mixtures included each lucerne type associated with each grass species and a complex (4-component) mixture with both lucerne types and both grass species. Perennials were only evaluated in Sassari and Marchouch. Annuals were harvested once in spring with legumes at early pod-filling and cereals at heading stage. Results of annual crops across three sites and two evaluation seasons are summarised in Table 3.

Table 3. Mean values of crop dry-matter yield (DMY), weed proportion on total dry biomass, legume proportion on crop DMY, land equivalent ratio (LER), and visual appraisal score (scale 1-5) attributed by local farmers, for different forage crops across two growing seasons in three Mediterranean sites (farmers' score from only two sites). Cereal data in PS and BM are the averages of crops including oat or triticale. Elaboration from Annicchiarico *et al.* (2017a)

Crop	DMY crop (t/ha)	Weed %	Legume %	LER	Farmers' score
PS – Cereal	6.26	6.7	–	–	3.50
PS – Tall pea	5.85	8.6	–	–	3.76
PS – Semi-dwarf pea	5.29	12.9	–	–	4.39
PS – Common vetch	3.82	12.9	–	–	3.60
BM – Cereal + tall pea	6.23	7.2	40.4	1.03	3.93
BM – Cereal + semi-dwarf pea	5.60	9.2	32.9	0.97	4.03
BM – Cereal + common vetch	5.56	5.6	33.2	1.02	4.01
CM – 2 cereals + 2 pea types	5.45	7.1	37.3	0.92	3.57

PS: pure stand; BM: binary mixture; CM: complex mixture.

The tall pea line showed the greatest competitive ability versus the cereals and maximised the yield and legume proportion of legume-cereal crops (being significantly superior to the semi-dwarf pea line). Vetch-cereal binary mixtures had lower weed content than the average of the component pure stands. Cereal pure stands (particularly oat) were top yielding but modestly appreciated by farmers. Pea monocultures combined good yielding ability, competition against weeds and farmers' appreciation. The latter trait, however, was very site specific, suggesting that other factors besides crop productivity and competitive ability influenced the farmer perception of the crop value. Farmers in Sassari, for instance, tended to give greater appreciation to the widely known common vetch-

based crops, while crops including the semi-dwarf pea line were praised more as a potential grain crop than as a forage crop, and the fear of possible difficulties in the harvest penalised the tall pea line despite its good performance. No differences among crops were found for the Land Equivalent Ratio (LER) of mixtures. Cereal-tall pea and cereal-common vetch binary mixtures had LER values above the unit, indicating a mild, but positive, effect of species complementarity compared to the respective pure stands.

Forage quality of binary and complex mixtures of annual crops was assessed over two growing seasons in Sassari (Porqueddu *et al*., 2016b). The common vetch-based binary mixtures had higher protein content than the pea-based binary or complex mixtures, whereas no differences were observed for NDF, ADF or digestible dry matter (DDM). NDF and ADF values allowed to classify all mixtures as good (NDF < 500 g/kg; ADF < 310 g/kg). DDM was quite good in all mixtures ranging on average from 616 to 632 g/kg across mixtures. Triticale was invariably less competing than oat in mixtures, resulting in a positive effect on the forage quality of its binary mixtures in terms of protein, digestibility and fibre, regardless of the companion legume.

For perennials, DM production was mostly concentrated in spring in Morocco (5 cuts across three years), whereas the crops produced throughout the years in Sardinia (11 cuts) but with lower yield. The cumulated DM yield of perennials averaged across the two evaluation sites showed that the erect lucerne cultivar Mamuntanas (alias Surigheddu) in pure stand yielded 127% more forage, and the semi-erect type 53% more forage than the mean of the two grass pure stands (the adapted cultivars Flecha and Kasbah). The lucerne-grass binary mixtures did not increase the DM yield compared to the respective lucerne pure stands. The complex mixture outyielded the pure stand and binary mixtures of the semi-erect lucerne (+42%) but not those of the erect lucerne variety. Consistent with the productive results, lucerne in pure stand displayed greater farmers' acceptability than lucerne-grass mixtures.

The reported comparison between annuals and perennials in the same location and the same period (Annicchiarico *et al*., 2017b) indicated that annual crops outyielded perennials (6.35 *vs* 5.14 t/ha) and were more competitive against weeds (3% *vs* 26% weed proportion, respectively). However, the DM yield of the perennial complex mixture was as high as that of the best annual crops. On average, the proportion of legumes in mixtures was high both in annuals (40%) and perennials (53%). The advantage of mixtures over pure stands was much greater for perennials than for annuals in terms of LER (1.31 *vs* 1.03). As the yield efficiency of a mixture, hence its LER, is mainly determined by the performance of its weaker component, the low yield potential of the grass pure stands accounted for such a relative advantage of mixtures in perennials.

VII – Conclusions

The large set of experiments performed for almost 15 years in the western Mediterranean basin provided evidence that germplasm adapted to dryland conditions can be found in all main forage species. These genetic resources could be exploited for direct introduction into cropping or as useful parental material in breeding programmes. With the possible exception of the best tall fescue germplasm, in all other species outstanding genotype × environment interaction (with inconsistent cultivar ranking across environments) was found for yield, reinforcing the need for selecting specific varieties for each sub-region. Specific selections in the main species are on their way or produced already candidate varieties. The more or less direct interface between the research and the productive world that the long period of experimentation enabled brought to attention a very important practical hindrance to the adoption of novel, adapted forage crops, that is, the general weakness of the forage seed chain in the Mediterranean region.

A key outcome of this activity is that legume-based crops displayed higher forage quality and farmers' appreciation than pure-stand crops of cereals or grasses. Pea showed much greater potential as a forage crop for Mediterranean environments than hitherto believed, both in pure stand and in intercropping. Common vetch was about as valuable as pea for mixed cropping. Lucerne mixtures with grasses were high yielding, but pure stands of adapted germplasm yielded as much and were appreciated by farmers. Complex mixtures did not provide a clear advantage over binary mixtures. In general, the best annual crops tended to be higher yielding than the best perennials and may be preferable for hay production. However, perennials offer the advantage of extended herbage production, particularly in less stressed environments.

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