The European Journal of Plant Science and Biotechnology ©2011 Global Science Books

Physiological and Bioagronomical Aspects Involved in Growth and Yield Components of Cultivated Forage Species in Mediterranean Environments: A Review

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ABSTRACT

This review provides an updated perspective on the effect of agronomic management and weather conditions on biological pathways in the organs of plants used for herbage seed production. Biochemical pathways from seedling to herbage harvest or seed production in forage crop species are emphasized. The review reports the effect of weather conditions and agronomic practices on physiological processes in plants growing during forage and seed yield production in Mediterranean or Mediterranean-like environments. When the available scientific information is not adequate to explain the physiological aspects involved in plant development, experimental results on forage crops or related species in environments close to the weather condition of a Mediterranean climate have been used. Plant breeding progress of the agronomic characteristics seed and herbage production achieved in annual and perennial forage crop varieties included in the Italian register of the EU catalogue, in the last 10 years, is scant in comparison to those of other cultivated crops. The genetic gain in forage crop varieties for herbage production over this period has been 4% in herbage against 13.0% in cereal grain crops while no breeding progress has occurred for seed production and seed yield components. Knowledge of the effect of agronomic management and weather conditions on physiological processes in plant organs during the growing cycle of herbage and seed production is useful to develop a breeding strategy able to endow the biochemical requisites to better exploit harsh weather conditions.

Keywords: adaptation, agronomic management, forage crops, herbage and seed yield, physiological processes

Abbreviations: ADP, adenosinediphosphate; **AGC**, allelometric growth coefficient; **ARS**, adventitious root system; **cDNA**, complementary deoxyribonucleic acid; **DAP**, days after pollination; **GA**, gibberellin; **HI**, harvest index; **HMW**, high molecular weight; **LD**, long-day photoperiod; **LMW**, low molecular weight; **MDH**, malate dehydrogenase; **mRNA**, messenger ribonucleic acid; **NADP**, nicotinamide adenine dinucleotide phosphate; **NSC**, non structural carbohydrate; **OAA**, oxalacetate; **QTL**, quantitative trait locus; **PEP-C**, phosphorenolpyruvate carboxylase; **3-PGA**, 3-phosphogliceric acid; **PGR**, plant growth regulator; **PTH**, high photoperiod and day/night temperature (16 h – 25°C/20°C); **PTL**, low photoperiod and day/night temperature (10 h – 20°C/18°C); **SD**, short-day photoperiod; **SEM**, standard error of the mean; **Rubisco**, ribulose 1.5-ribulosephoshate; **WSC**, water-soluble carbohydrate

CONTENTS

GROWTH AND YIELD COMPONENTS IN CULTIVATED CROPS

The potential genetic background in a cultivated forage plant depends on the influence of morphological and physiological plant characteristics affected by weather factors during the growth period. Biomass production and its repartitioning in sink organs are related to the interrelationships among physiological processes. Biological processes of biomass repartitioning into vegetative and reproductive parts of the plant underlie the complex framework of phytomass production in plants, mainly in forage crop species (Wardlaw 1990; Wilhelm 2002; Avice *et al*. 2003; Sadras and Egli 2008).

The physiological processes adopted by plants to capture and store solar energy in useful products for humans and livestock feeding consumption are revived. A critical description of some biological processes which promote partitioning of the accumulated energy within plant parts helps to better understand the pathways involved in plant growth processes. Nowadays, the availability of sophisticated techniques for investigating metabolic processes in the organs of plant represents tools to better study metabolic transformation able to increase solar energy conversion into phytomass (Wilhelm 2002; Hay and Porter 2006; Zhang and Wang 2007). The potential biomass in forage crop production depend on the leaf morphological structures and canopy, mesophyll cell size, leaf senescence, leaf endow-

Received: 3 February, 2009. Accepted: 2 February, 2011.

Invited Review

Table 1 Effect of tiller biomass whose removal is based on phenological stage (Elaborated from: Iannucci *et al.* 1996).

Growth Stage	Leaf			Stem			Root		
	CP[a]	**GL**[b]	**RS**[c]	**CP**[a]	**GL**[b]	**RS**[c]	**CP**[a]	**GL**[b]	**RS**[c]
Stage I	31.0	5.1	3.8	16.6	4.9	3.1	14.8	5.8	2.1
Stage II	28.2	5.8	5.0	12.2	11.6	3.9	14.1	6.2	2.2
Stage III	25.1	6.3	4.2	10.1	6.5	4.4	12.2	6.4	2.0
Stage IV	19.7	5.2	4.3	9.8	3.0	1.9	10.2	2.8	1.6

[a] % Crude protein
[b] % Glucose
[c] % Reduced sugar

ment and sink control of the plant (Durand *et al.* 1991; Wright 2007).

During plant growth, flowering induces variation of assimilates mainly in the stem which, before flowering, is a temporary sink organ of biochemical compounds. The stored physiological products of the overwinter vegetative stage of plant development at the preheading stage are related to the species of forage crop. The stored compounds of the roots, stems and leaves at the blooming phenological stage were relocated during the grain-filling period to fructiferous organs of the plant. The allometric relationsphips between rate and duration of physiological mechanisms which the relocations of compounds in the vegetative and reproductive organs undertake are genetically controlled and are strongly influenced by weather conditions (Heide 1994; Avice *et al.* 2003; Sadras and Egli 2008; Zhao *et al.* 2008). However, in crops where seed is the most important product of agronomic cultivation, the physiological process has been the object of genetic selection to increase the efficiency of relocation of biochemical compounds. In contrast, in forage crops, where the vegetative parts of plants are the main agronomic concern, breeding efforts to improve the efficiency of relocation for biomass and seed production are still neglected (**Table 1**) (Claser *et al.* 2000; Barret *et al.* 2005; Baezinger *et al.* 2006).

The purpose of this review is to focus attention on the influence and on the effect of environmental factors on biological routes of chemical compounds. By highlighting the main research on relocation in the organs of plants during the vegetative and reproductive phenological phases of growth will provide knowledge for improving the adaptability of forage crops.

Edaphic environment of plant growth

Most regions of the world characteristically have limited environmental factors which interfere with physiological mechanisms of plant growth and reproduction causing a reduction in the yield potential of the gross product (Iannucci *et al.* 2000b; Jabbar *et al.* 2003).

Meteorological events, mostly diffused in land with agricultural interest, are low precipitation and high temperature, which mainly determine water deficit during plant growth and reproduction. Water is the major factor limiting crop growth in agricultural land, 31-36% of which receives 50-310 mm of annual rainfall while the remaining 64-69% has a temporary limited period of water stress during the growth season. The effect of moisture stress is a complex interaction among soil and atmospheric factors which control water use efficiency (WUE) and physiological metabolisms during the growth of plant roots, stems, leaves and seeds (Iannucci *et al.* 2000b; Schoonhoven *et al.* 2006; McAdam and Barta 2007; Jahufer *et al.* 2008).

In annual and perennial forage crops, weather conditions influenced phytomass and seed yield and alter the nutritive value of the biomass. In the literature, mainly in cereal crops, there are several reviews on various aspects of grain crops and plant growth in extensive systems while in forage crops, information on biomass and seed yield components are very scant (Claser *et al.* 2000; Humpheys 2005; Baenziger *et al.* 2006).

SEEDLING DEVELOPMENT

Seed germination in soil is a critical phase characterized by having its own growth-limiting factors and responses to environmental conditions. Seedling development encompasses several distinct stages of growth: heterotrophic, transitional and autotrophic (Cooper 1977; Meyer 1999). Water availability and temperature of the soil surface are the factors which mainly affect seed germination of seedling establishment (Iannucci *et al.* 2000a; Venuto *et al.* 2002).

The energy required to sustain the beginning of the life process, in the heterotrophic stage of seedling growth, depends upon seed quality of storage reserves in cotyledons and the endosperm. The amount and quality of stored compounds provides nutrients to the embryo for developing hypocotyls. Thus, species with small seeds present low seedling emergence and, as a consequence, major vulnerability to edaphic and environmental stresses than species with large seeds. Seminal roots of seedlings originate in the soil from germinated seeds. This kind of root supplies for a short time, in eight species of perennial grasses (thickspike wheatgrass, desert or crested wheatgrass, western wheatgrass, altai wildrye, Russian wildrye, little bluestem, sideoats grama and blue grama), water and nutrients for the developing seedling, but only to the hydraulic conductivity system of coleoptiles (Redmann and Qi 1992; Venuto *et al.* 2002).

In the heterotrophic phase, the energy needed for the transitional stage of seedling growth is supported by chemical and biochemical compounds stored in the seed cotyledons whose amount is related to the seed reserve. Seed planting depth, soil temperature (optimum near 20°C) and field capacity moisture content are the physical conditions which facilitate germination and indirectly save or reduce energy for the heterotrophic phase of growth (Jensen *et al.* 1972; Redmann and Qi 1992; Iannucci *et al.* 2000a; Venuto *et al.* 2002).

The photosynthetic activity of seedlings begins after the emergence of hypocotyls. These events happen before depletion of the nutrient reserve while mineral absorption initiates at the end of the heterotrophic stage (Kedall and Striger 1985; Meyer 1999). Thus, root activity in seedlings, at the end of the heterotrophic phase, prevents photosynthesis (Alcordo *et al.* 1991; Redmann and Qi 1992).

The morphological point of ARS development is the subcoleoptile internodes. Studies carried out by Tischler and Voigt (1993) in panicoid grasses (sideoats grama, blue grama and klein grass) and Sakanoue (2004) in red clover in a mixed-sown meadow found that the growing point of the ARS originated from seedling coleoptiles internodes and their development was strictly related to the species of forage crop.

The agronomic association among legumes and grasses forage crops favours genotypic interaction in a plant mixture whose root system leaves biochemical compounds in the soil which favour an increase in qualitative and quantitative biomass production (Zannone 1985; Zannone *et al.* 1986; Martiniello 1999). In a study carried out in France by Gallais (1970) on binary mixtures between grasses and legumes, he developed a model which evidenced that genotypes that are more productive in a pure stand are also more productive in mixtures. The intergenotypic interaction in a plant mixture was discovered by Turkington (1996) using a model system which evidenced that the advantages achieved in the association between genotypes of perennial ryegrass and white clover was ascribed to soil microbes which favour a higher root system activity than pure stands.

However, the ecological complexity among intergenotypic, agronomic and environmental interactions causes difficulty in discovering the biochemical compounds which result in the agronomic advantages. In field experiments carried on agronomic cultivation of crops in pure stands and in associations, Martiniello (1999) discovered that four clovers-Italian ryegrass and a clovers-barley binary mixtures increased herbage production yield by 106% under rainfed and 162% under irrigated condition. Analogous results were found by Piano and Annicchiarico (2008) in white clover-grasses which increased dry matter by 24% in comparison to crops in pure stands. Furthermore, Pala *et al.* (2008) in a field experiment in semi-arid Mediterranean environments found that the vetch-barley association advantaged dry matter yield of both crops and the effect of agronomic practices.

The full establishment of an ARS (complete development and size of morphological structures) is related to the moisture and temperature of the soil. The time required for seedling establishment, in semi-arid environments, is about 2 months. However, harsh weather conditions reduce the development of the ARS and when stress conditions are persistent, the seedling dies (Mayer 1999). The biological functions of a seedling outlives its activity (generally after 3-5 days after germination) when the coleoptiles tip intercepts and transmits light to the phytochrome system of the crown. At this moment, the seedling and root begin the autotrophic stage of development, taking up nutrients from the soil and creating photosynthates from photosynthetic activity (Tischler and Voigt 1993; Meyer 1999; Stephen *et al.* 2006). The autotrophic stage of seedling development is related to seed size. Seedlings that develop from small seeds under low moisture conditions in the soil are less susceptible to stress than those derived from large seeds (Black 1958; Muir *et al.* 2005). Furthermore, Anderson (1971) in diploid and tetraploid red clover reported that the relationship between seed size and seedling growth is not affected by ploidy level while petiole length and leaf area of a diploid plant are smaller than those derived from tetraploid seedlings.

Transition of seminal to ARS is a critical stage for seedling development. The passage from autotrophic to physiological function of the seedling is linked to the availability of adequate soil moisture for about one week. During this phase, if water in the root zones is not available, the development of the ARS fails and the seedlings die (Newman and Moser 1988; Ta *et al.* 1990; Tischler and Voigt 1993; Wilhelm 2002). However, differences in seedling development were found between C_3 and C_4 plants. The 63% of lower mortality observed in C_3 more than C_4 seedlings under water stress development, particularly in large seeds was ascribed to seedling vigour and morphological reliance of seminal roots for water uptake (Qui and Redmann 1993; Wilhelm 2002; Sadras and Egli 2008).

Allelopathic effects

Development of a root system leaves biochemical components in the topsoil which can promote positive or negative allelopathic effects on a plant's root growth. The toxic substances in the soil which promote allelopathic effects are phenolic acids, alkaloids, cumarins, tannins, flavonoids, steroids and quinines (Einhelling and Leather 1988; Khanh *et al.* 2005). These compounds are considered to be degradation products (secondary metabolites) of isoflavonoids or related compounds (Rice 1986; Turkington *et al.* 1977; Golisz *et al.* 2008).

Laboratory experiments with legumes (mainly clovers) and grasses well diffused in grasslands evidenced that extract or lactates of some seedlings inhibited seed germination or seedling growth of the same and other species (Larson and Schwar 1980; Peters and Mohammed Zam 1981; San Emeterio *et al.* 2004).

A field experiment, established to assess allelopatic effects, was conducted by Larson and Schwarz (1980). The authors used forage grass infected by fungal entophytes (*Neotyphodium coenophialum* Morgan-Jones and Gams). Mulch formed with grass clippings was used to generate an infected plant status. There was allelopathic inhibition of root development in legume and grass species and a significant increase in root nodules grown on mulched fields. A similar significant reduction in root and shoot weight was found in studies by Peters and Zam (1981), Springer (1996) and Sutherland *et al.* (1999), on germination of clover seed in leaf extracts of tall fescue. The authors attributed the decrease of biomass production to an allelopathic effect on clover by grasses (perennial ryegrass and tall fescue) and variation observed among seed germination was exclusively attributed to legume sources rather than fungal endophytes present on grasses. Similar information was collected in wimmera ryegrass by San Emeterio *et al.* (2004) in a greenhouse experiment on normal and fungal endophyte-infected plants. The authors evidenced that reduction of seed germination, seedling growth and an ARS was due to chemical compounds released by the root system of the plant and not by the fungal agent.

However, there is no evidence between toxic compounds discovered in the laboratory and those observed in the field. In forage crops, the threshold level of phenolic acid compounds which hindered the allelopathic effect in the laboratory is specific and in the field has not been discovered (Rice 1986; Springer 1996; Iannucci and Di Fonzo 2005). An experimental association carried out under controlled environments by Iannucci and Di Fonzo (2005) and Iannucci (2007) on two species of grasses (Italian ryegrass and oats and five species of legumes (California burclover, berseem, crimson clover, squarrosum clover and common vetch) reduced, in comparison to the pure stand control, the development of seedlings and roots of legumes by 18.5 and 15.5%, respectively. The legumes species studied differed in their response to the oat and Italian ryegrass root exudates whose allelopathic effect influenced root and seedling elongation of legume species differently. The association of both donor grasses, between crimson clover and California burrclover, was more tolerant to the allelopathic effect than common vetch and squarrorum and berseem clover. The authors noted that the elongation effect achieved by allelopathic compounds on root and seedling organs of species was specific: Italian ryegrass reduced root and seedling elongation in vetch and only the root in berseem and squarrosum clover.

Sutherland *et al.* (1999) and Khanh *et al.* (2005) reported that the effect of allelopathy, which arose from an association among grasses and legume forage crop species on plant development, plays an important role for establishing a sustainable agriculture. This was attributed to the fact that exploiting the allelopatic effect, induced by a donor plant (crop grown) during crop cultivation, reduced the development of biotic or abiotic agent (disease or weed) and, as a consequence, favoured the reduction of external input for their control.

The allelopathic effect from crop associations benefits the establishment of a natural biological mechanism inside a crop that is able to govern biotic and abiotic agents, which usually requires considerable external input for their control in agronomic production (Olofsdotter *et al.* 1995; Macias *et al.* 2001; Mamolo and Kalburtji 2001).

Exploitation of the allelopathic effect in a large-scale cropping system represents an agronomic opportunity for developing natural biological compounds in the soil able to establish environmental friendly and beneficial sustainable agriculture (Xuan *et al.* 2005). Information on farming agronomic exploitation of allelopathic properties in plant associations reported by Om *et al.* (2002) and Golisz *et al.* (2008) in soft wheat-rice and buckwheat and Miller (1996) and Schroeder (2006) in lucerne-wheat evidenced a wide range of associations endowed with the potential to reduce mainly the content of weeds and pathogens in cultivated crops. However, the authors underlined the ecological importance of crop associations for biological control of weeds and fungal diseases and recommend the need to further study the physiological pathways of the allelopathic effect.

ROOT DEVELOPMENT

After seedling establishment, the development of root systems depends on soil fertility, weather conditions and agronomic utilization of the aerial part of the plant for agronomic consumption (Newman and Moser 1988; Kertulis 2001).

The root, after defoliation, stops to grow, fine roots of the ARS die and the taproot reduces development. Defoliation in forage crops allows a cyclic pattern of starch degradation followed by reaccumulation in taproots (Hadden and Volenec 1990; Donaghy and Fulkerson 1997). Electron microscopy investigation carried out by Luo *et al.* (1995) on taproots of field-grown lucerne after defoliation, in the first week of plant regrowth evidenced no starch grains in the centre of taproots. During the third and fourth weeks of regrowth a uniform increase of starch grains in the cells of root rays was found, but by 28 days after defoliation the distribution of starch grains in the taproots become normal: more starch grains in ray cells near the cambium zones than near the centre of the taproot. The intensity of root damage depends on the frequency and severity of defoliation. The content of root dry weight, total nonstructural carbohydrate, soluble sugars and crude protein, across harvest of aerial biomass at flowering plant phenological phases of development of bermudagrass, berseem, birdsfoot trefoil and lucerne, are strongly reduced with defoliation when compared to those of unclipped controls (Alcordo *et al.* 1991; Boyce *et al.* 1992; Martiniello *et al.* 1993; Iannucci *et al.* 1996; Rotili *et al.* 1996).

The root-system biomass development in berseem, bermudagrass, lucerne and white clover has been shown to be positively related to the amount of evapotranspiration, crop hydrotropism and negatively to the aerial biomass exported by defoliation (Abdul-Jabbar *et al.* 1982; Martiniello *et al.* 1993; Tsutsum *et al.* 2003). Soil temperature is the critical factor of a root system's development and storage of reserve compounds. Maximum development of root growth for dry matter accumulation, N_2 fixation and total nonstructural carbohydrate occur when day temperature is around 21-23°C. A night temperature of 19°C favours taproot development while 4°C promotes lateral root expansion and depressed taproot development and physiological activity of total non-structural carbohydrates (Dady and Williams 1976; Sato and Itoh 1974; Munns *et al.* 1977; Seker *et al.* 2003). Drier rather than mild soil moisture leads to deeper and more branched rooting systems than those developed under soil-stressed conditions (MacDuff and Jackson 1992; Wang *et al.* 2004; Jahufer *et al.* 2008).

The effect of photoperiod on the root system under field experiments is not well documented. However, experiments study carried out in a controlled environment in white clover and Italian ryegrass by MacDuff and Jackson (1992), Goulas *et al.* (2002), Thomas and Hay (2008) and Jahufer *et al.* (2008) reported that the effect of photoperiod influenced the metabolism of N routes and the morphological pattern of root system development. Moreover, the effect of a 12-h vs 8-h photoperiod reduced root growth while the exposure of 14 vs 11 h of photoperiod did not affect development. The effect of photoperiod on seedlings for 12 h vs 8 h reduced root system development, increased root branching and enhanced axillary buds at new sites of leaf production during the period of regrowth. Extending the photoperiod for 14 h vs 11 h, however, did not influence root development and reduced the content of cytokinin in roots and leaves (Mannetje and Pritchard 1974; Hesterman and Teuber 1981). The difference between the two light exposures plays a role on the ratio distribution of dry biomass between total plant and root system.

The effect of defoliation on perennial forage crops: cocksfoot, perennial ryegrass, harding grass and tall fescue reduced root growth and the proportion of N in root and shoot regrowth. All species showed morphological adaptation of the organs which potentially increased their adaptability to tolerate defoliation (e.g., increased allocation of N to shoot at expense of root and lower specific leaf weight). However, particularly in lucerne the responses of plants to defoliation reduces radiation efficiency, accumulation of carbon and N partitioning in roots and shoots, nodule activity in N_2 fixation, uptake of nutritive values altering the metabolic pathways of plant growth (Mackie-Dawson 1992; Richards 1993; Sanderson *et al.* 1997; Teixeira *et al.* 2007).

The regrowth dynamics after defoliation of the stored carbohydrates and N compounds stored in the root systems plied an important role during plant regrowth. Results obtained in prairie grass and perennial ryegrass showed that water soluble carbohydrate and N compounds in stubble and root reserves supplies the energy requirement by compensatory photosynthesis (photosynthesis of remaining green organs not removed with defoliation (stubbles, leaves and active mersistems) of the plant until the appropriate photosynthetic tissue had been produced. The sequence for allocation of soluble carbohydrates and N reserves are utilised for leaf development, root growth and tillering during the regrowth period. The water-soluble carbohydrate reserves were identified as primary contributors to plant regrowth after defoliation by providing energy to rebuild biological processes for recovering the stored compounds (carbohydrates and protein) through metabolic adjustment of the ARS and leaf photosynthetic area (Behrenfeld *et al.* 1998; Rawnsley *et al.* 2002; Mehaffey *et al.* 2005; Lasseur *et al.* 2007; Turner *et al.* 2007).

The agronomic techniques regarding cutting height of herbage biomass which safeguard the active meristems present in the crown of lucerne, birdsfoot trefoil, red and white clovers, avoid or reduce a stressed root system and favour compensatory photosynthesis for rebuilding the photosynthetic area, increase the quality of biomass and persistence of forage crops (Nowak and Caldwell 1984; Buxton *et al.* 1985; Dong and de Kroon 1994; Wiersma *et al.* 2007). Martiniello (1992b), Dong and de Kroon (1994) and Turner and Pollock (1998) in studies made on the height of harvest in rhizomatous plants (both grasses and legume crops) found that cut height during defoliation (8-10 cm from above ground level) saves the stolon nodules located at the base of shoots near or slightly below the soil surface and maintains active meristems of axillary buds reducing the effect of stress of regrowing stems. Therefore, in lucerne, accurate and appropriate management practices during harvest saves axillary buds of the root crown and minimizes plant stress after defoliation (Wiersma *et al.* 2007).

VEGETATIVE GROWTH

Aerial phytomass is mainly related to the environmental effect on plant development. Brueland *et al.* (2003) reported that each phenological stage of growth is characterized by optimized temperature during plant development. Environmental factors like light, temperature and moisture play an important role in the efficiency of basic physiological processes. Light interacts with other environmental factors (mainly water and temperature) influencing the carbon exchange rate and relationship with phytomass production (Hart *et al.* 1978; Wilhelm 2002). Thus, biomass yield is a function of vegetative growth rate and plant morphology characteristics (plants per unit area, number of shoots per plant and shoot weight). The number of plants per unit area depends on the species and on the sowing seeding rate (Pulli 1980; Martiniello and Ciola 1995; Sulas *et al.* 2000). Soil moisture, solar radiation and evapotranspiration are the environmental factors which mainly influence the number and development of stem density (Perry and Larson 1974;

Martiniello *et al.* 1997; Iannucci *et al.* 2002). The maximum number of stems, in the same environment and growing condition, is usually attained before heading in both defoliated and undefoliated plants. The decline of stem number begins when the plant reaches the fully canopy (Martiniello *et al.* 1997). Defoliation, in both annual and perennial crops reduces, as an effect of plant mortality, the number of stems per unit area. In experiments carried out in Mediterraen environments the effect of defoliation on stem mortality in regrowth was found to be more than 10% of the previous harvest annual (Italian ryegrass, crimson clover, berseem and Persian clovers) and 15% in perennial (cooksfoot, lucerne sulla, sainfoin and tall fescue) forage crops (Martiniello and Ciola 1994; Martiniello *et al.* 1996; Martiniello and D'Emilio 1997; Martiniello 1999; Sulas *et al.* 2000; Martiniello 2009).

Defoliation made during the growing season reduces stem density and stem weight. Investigation on the effect of defoliation on biomass provides information on the relationships among phytomass yield components traits. Thus, a study of yield components allows the possibility to better understand the effects of environment on plant development and metabolic processes of stored biological compounds in morphological characters of forage crop species grown in environments with Mediterranean climates (Martiniello *et al.* 1996; Iannucci *et al.* 1996; El Karamany 2006; Teixeira *et al.* 2007).

Plant development

Phytomass production depends mainly on the genetic yielding potential of the crop and on the edaphic adaptation to environmental factors with crop management (**Table 2**). In forage crops, the respiration process after defoliation of the above-ground phytomass, in both annuals and perennials, applies a strong stress on the root system, and the rhizome and stolon deprive, partially or totally, the stored compounds in the sink organs of the plant. In perennial forage crops, Martiniello *et al.* (1997) in lucerne, Cheplick and Chui (200) in timothy, Belesky *et al.* (2006) and Turner *et al.* (2006a) in cocksfoot and Volesky and Anderson (2007) in meadow bromegrass and creeping meadow foxtail found that the intensity of the environmental stress condition during the regrowth of stems after defoliation influenced the relocation of photosynthetic compound in the stored organs and phenological stage of plant development.

Defoliation in legumes (lucerne, subterranean clover) and red clover) heavily reduced N fixation in the first 6 days of stem regrowth by the legume-*Rhizobium* symbiosis causing a reduction in N compounds in the nodules supplying the above-ground demand for stem development of N biochemical products (Hendershot and Volenec 1993; Corre *et al.* 1996; Scotti *et al.* 1997; Schulze 2004).

A study made in forage legume, plant regrowth in the first 6 days after defoliation evidenced a reduction by 50-55% of the initial aminoacid and storage proteins. Legume species have a limit of N sink strength which represents a trigger of N feedback mechanism which regulates N activity through variation of the oxygen diffusion resistance in the nodule reducing the activity of the nitrogenase enzyme of the *Rhizobium* (Richards 1993; Hartwig and Nösberger 1994; Kang and Brink 1995; Schulze 2004).

On experiments based on the defoliation interval of cocksfoot, berseem, lucerne and white clover evidenced that the compensatory photosynthesis of the remaining green surface after defoliation began to recover the water soluble-carbohydrate, N energy reserve status, metabolic adjustment in the organs of a plant and regrowth of stems, leaves and roots. (Hartwig *et al.* 1987; Richards 1993; Kangand Brink 1995; Frugier *et al.* 1998; Martiniello *et al.* 1996; Iannucci *et al.* 2000b; Turner *et al.* 2006b).

Table 2 Water use efficiency in forage crops in two years experiments established under alternative condition of water supply in Mediterranean environment (Elaborated from: Martiniello 1999 annual clover legumes, and perennial crops by Martiniello 1998a).

Species of forage crops	Water Use Efficiency ^{a}	
	Rainfed (± SEM)	Irrigated (± SEM)
Annual legumes		
Berseem clover	11.7 ± 1.1	10.2 ± 1.3
Crimson clover	10.9 ± 1.6	8.6 ± 1.6
Persian clover	10.9 ± 0.9	8.5 ± 0.9
Squarrosum clover	12.5 ± 1.1	12.0 ± 1.1
Perennial crops		
a) Legumes		
Lucern	16.3 ± 1. 2	14.1 ± 2.1
Sainfoin	19.9 ± 1.4	10.1 ± 1.8
Sulla	22.9 ± 1.2	9.2 ± 1.8
b) Graminaceous		
Cocksfoot	24.7 ± 2.7	10.3 ± 1.9
Perennial ryegrass	30.3 ± 2.4	10.1 ± 2.1
Tall fescue	24.3 ± 2.3	11.0 ± 1.1

^{a} kg dry matter ha^{-1} mm^{-1} water

The photosynthetic activity in the rebuilt organs of plants depends to the morphological and physiological characteristics of the forage crop, to the amount of remaining meristems after defoliation (mechanical harvest or grazing) and to the available weather resources. An investigation made by Iannucci *et al.* (1996) on the content of crude protein, total soluble and reducing carbohydrate in roots, stems and leaves of berseem under defoliation applied in four different phenological stages of plant development (elongation of the 4th and 8th internode, early flowering and physiological maturity of seed), evidenced that crude protein declined in all three organs passing from the fourth node to physiological maturity; total soluble and reduced carbohydrate in the first until third stage of development remained constant while all compounds in the fourth stage of development dropped. A further investigation made on the same species by Iannucci *et al.* (2000b) evidenced that leaf water potential, osmotic potential, gravimetric water and leaf proline content were influenced by hardening and the available weather resource (mainly moisture). The increase of water deficit reduced the content of the parameters while acclimatization contributed to reduce the effect of water stress on plant tissues.

In field experiments made on cocksfoot under different Australian environmental weather conditions by Rawnsley *et al.* (2002) and Sinclair *et al.* (2006) found that the environmental condition of the trials influenced the content and distribution of the nonstructural carbohydrate and crude protein among the plant organs of defoliated cocksfoot, perennial ryegrass, prairie grass and tall fescue. Both authors investigated cocksfoot whose treatment was based on defoliation interval and leaf stage. They found that defoliation of cocksfoot plants resulted in reduced water-soluble carbohydrate assimilation and therefore leaf, root and stem dry matter accumulation during the subsequent recovery period. Their results showed that defoliation based on development of the leaf stage maximized water-soluble carbohydrate reserves, tillering and leaf and root dry matter yield. The priority sequence of the allocation of water-soluble carbohydrates in the organs of plants during the regrowth period was in the order: leaf, root then tiller growth. The new N energy reserves after defoliation played a minor role in the regrowth of cocksfoot.

Avice *et al.* (2003) in field experiments carried out in the vegetative stage of plant development of lucerne and white clover after defoliation noted that the root storage proteins were extensively mobilized to satisfy the N requirements of tiller regrowth. These authors saw the environmental influence on the quality of protein synthesis during the vegetative stage of development which hardened the species to tolerate weather stress conditions. These observations suggest that storage proteins after defoliation do not exclusively serve as N reserves during the vegetative phase of legume development, but may play important adaptative roles in plant protection against abiotic (weather condition) and biotic (pathogen attack) tolerance (Iannucci *et al.* 1996; Rawnsley *et al.* 2002; Avice *et al.* 2003; Sinclair *et al.* 2006).

Since stoloniferous and rhizomatous grasses like Bermudagrass maintain many active meristems in axillary buds located on the stem and nodes of the stolon or rhizomes, stem regrowth resulted in greater tolerant to stresses caused by defoliation than brunchgrass with an upright *habitus* of development (Dong and de Kroon 1994; Martiniello 2008).

A study carried out on the effects of defoliation frequency on the number and viability of axillary buds by Suoto *et al.* (2004) on unquillo meadow grass of the Argentina's Patagonia grasslands. The field experiment was based on 1 to 5 defoliation treatments of grass compared with the undefoliated control. At the end of the growing season, plants with a high frequency of defoliation showed dead base stems and higher number of axillary buds at the periphery than the centre of the root crown in the unclipped controls. Furthermore, because the tissues in older buds of undefoliated tillers, the effect of senescence and deterioration of vascular bundles, showed lower metabolic activity than buds of defoliated tillers, the authors assumed that unquillo grass could tolerate the frequency of defoliation treatments without compromising its potential regrowth capacity and dry matter yield production. The variation observed in morphological and physiological activity of axillary buds in defoliated plants in a study carried out by Cheplick *et al.* (2001) and Belesky *et al.* (2006) in cocksfoot, perennial ryegrass and timothy was due to changes in the metabolic pathways of fiber, non-structural carbohydrate and protein biochemical pathways that occurred in the leaves and stems of tillers in response to external stress during vegetative growth which influenced the relocation of photosynthates in the storage organs of regrowth after defoliation.

Forage crops, both grasses and legumes, are characterized by a growing point of axillary buds above the soil surface that allows sensitivity to defoliation by mechanical management which may easily destroy the axillary meristems of the crown with a consequent deleterious effect on the future life of the plant. Thus, the morphological position of active meristems in the crown of a plant is important to consider during the defoliation process (Forde *et al.* 1989; Wiersma *et al.* 2007; Martiniello 2008).

Legumes forage crops with upright crown forms (as in lucerne) are more vulnerable to defoliation than those with the stolon forms near the ground level (as in white clover). However, in legume species grown for both biomass and seed production, the stems of the regrowth after defoliation showed better morphological performance (seed production and seed yield components) than those of non-defoliated plants (Martiniello *et al.* 1996; Martiniello and D'Emilio 1997; Iannucci *et al.* 2002). Martiniello (1992a) and Kang *et al.* (1995) in white clover, Martiniello *et al.* (1996) in berseem and Martiniello (1998a) in perennial legumes (lucerne, sainfoin and sulla) and grasses (cocksfoot, ryegrass and tall fescue) reported that plant defoliation affects stem regrowth and consequently seed production.

The agronomic cultivation of forage crops has to combine the development of advanced new engineering with technological processes and morphological characteristics of cultivated genotypes (root system, position of axillary buds) in such a way as to reduce the impact of management practice on metabolic activity of plant organs during the regrowth of stems for biomass or seed production (Volenec and Nelson 1994; Suoto *et al.* 2004; Wiersma *et al.* 2007; Martiniello 2009).

The agronomic management practices which take into account the form, growth and morphological position of meristems, reduce the stress of stem regrowth and increase biomass and seed production of the crops (Volenec and Nelson 1994; Rawnsley *et al.* 2002; Turner *et al.* 2006a; Wiersma *et al.* 2007; Martiniello 2009).

Stems

In forage crops, stem weight and internodes length are related to environmental conditions and available resources during the growing period. The stem development throughout the vegetative period of regrowth, in the absence of stress condition, remains nearly constant (Brown and Tanner 1983; Martiniello *et al.* 1997; Martiniello 2009). In experiments carried out by Martiniello and D'Emilio (1997) and Martiniello *et al.* (1997) between 1989 and 1997 in lucerne under alternative conditions of water availability, it was found that a reduction of soil moisture reduced the development of the stem (**Fig. 1**), induced changes in morphological structures (**Fig. 2**), and architecture of the plant (**Fig. 3**) and the leaf-leaflet area index for photosynthetic activity (**Fig. 4**). These results were in agreement with those obtained by Turner *et al.* (2006a, 2006b) in cocksfoot, perennial ryegrass and prairie grass and by Martiniello (2009) in lucerne, cocksfoot and tall fescue. Breeding studies made on berseem (Martiniello *et al.* 1992a), in lucerne (Jenczewski *et al.* 1999) and in soinfoin, sulla and harding grass (Martiniello 2005) assessed that environmental resources influenced the genetic characteristics of stem morphological traits. Stem weight, across the vegetative cycle, decreased with the number of defoliations made during the growing season and the above-ground phytomass was higher in spring defoliation than in autumn (Turner *et al.* 2006a, 2006b; Martiniello 2009).

Field agronomic experiments established in Mediterranean environments by Martiniello (1999) on perennial legumes (lucerne, sainfoin and sulla) and grasses (cocksfoot, ryegrass and tall fescue) under water irrigation treatments influenced dry matter and seed yield, stem density and height, seed per fructiferous organ and seed weight. Results showed a significant effect of irrigation treatments on stem regrowth, dry matter, seed production and their yield components. In legumes and grasses, a significant correlation was found between dry matter and seed yield with stem density and seed weight with seed yield in grasses while an opposite significant correlation was shown between seed yield and stems m^{-2} in legumes.

Bula (1972), Chatteron and Carlson (1981) and Wilhelm (2002) evidenced, in experiments carried out in lucerne under different environmental conditions, that temperatures of 24 to 25°C during the day and 18 to 19°C at night were optimal for plant development and phytomass production.

The effect of water stress was reported by Martiniello (1999) in annual clovers and grasses, Sulas *et al.* (2000) and Borreani *et al.* (2003) in sulla, and Martiniello (2009) in lucerne, cocksfoot and tall fescue, all of which reduced the growth of both stems and leaves. However, in cocksfoot, lucerne, tall fescue and big bluestem heavy stress condition (leaf water potential below 1.0 MPa) favoured development of leaves rather than stem growth; and stem development was more susceptible to moisture stress than other organs of the plant (Brown and Tanner 1983; Smart *et al.* 2001; Martiniello and D'Emilio 1997; Martiniello 2009).

Plant morphological form and number of stems in a unit area are the most important yield components for phytomass production (Sanderson and Reed 2000; Spinger *et al.* 2003; Springer and Gillen 2007). The maximum number of stems per plant in regrowth after defoliation was reached at 14 days after defoliation, and then declined as the canopy increased (Rawnsley *et al.* 2002). However, indirect measurement of morphological parameters, of biomass yield components like stem weight and leaf-stem ratio, are often assessed to better define the physiological process and nutritive value involved in crop growth (Frakes *et al.* 1961; Martiniello *et al.* 1996, 1997; Belesky *et al.* 2006). The establishment of stem density, in lucerne is related to seeding rate, environmental nutritive resources, plant health and phenological stage of the plant at defoliation (Martiniello *et al.* 1997; Guan and Nutter 2002; Martiniello 2009). In experiments established in a Mediterranean environment,

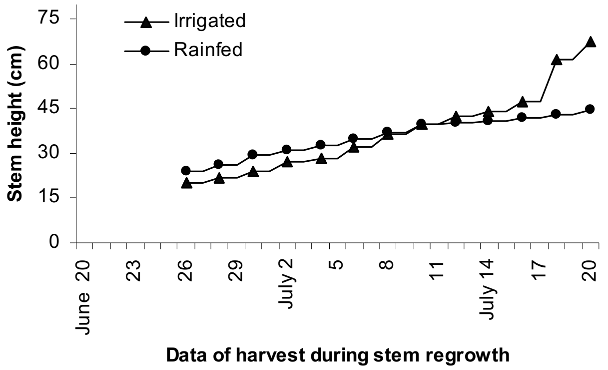

Fig. 1 Stem length during regrowth in cultivars of lucerne under rainfed and irrigated condition.

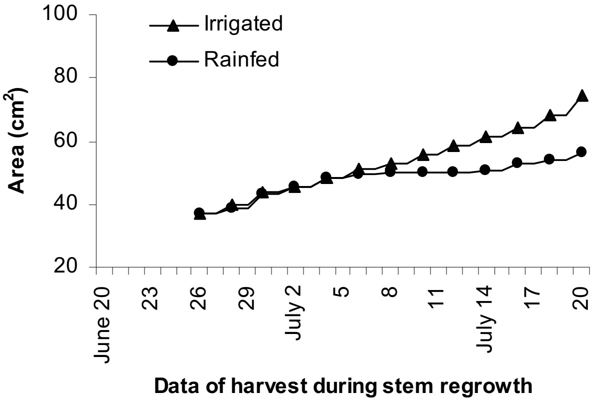

Fig. 2 Leaf area index of stems regrowth in cultivars of lucerne under rainfed and irrigated condition.

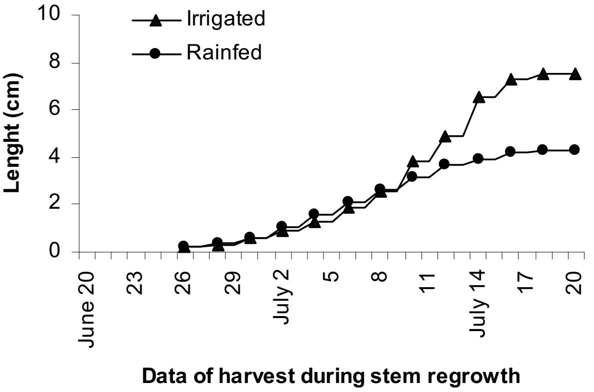

Fig. 3 Length of internodes in cultivars of lucerne under rainfed and irrigated condition of growing.

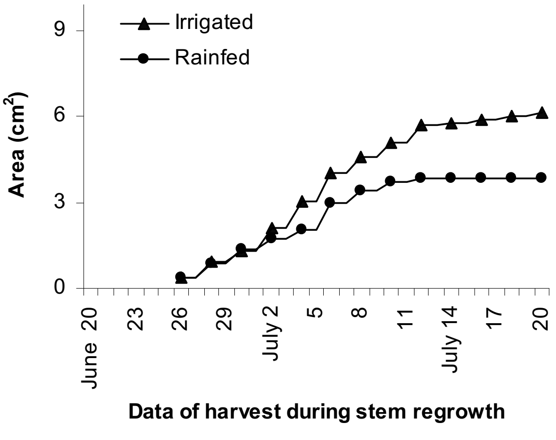

Fig. 4 Area of leaflets in leaf of regrowth stems in cultivars of lucerne under rainfed and irrigated condition.

based on seed sowing rate (high and low) and irrigation (irrigated and non-irrigated) treatments were detected the agronomic effect on performance, herbage and seed production on genotypes of common vetch, hairy vetch, field bean, berseem, crimson, Persian and squarrosum clovers and fenugreek (Martiniello and Ciola 1993, 1995), and sainfoin and sulla (Martiniello and Ciola 1994). Their results showed that seedling emergence in all these forage crops, the high seed m^{-2} sowing was higher than low seed m^{-2} rate. During the growth of annual legume crops, the stem density of the irrigated treatment was higher than the non-irrigated control. The low sowing seed rates in all forage species under non-irrigated treatment, positively affected forage and seed production while, except for hairy vetch, high seed rate increased herbage and seed production. In perennial legume crops, the difference between treatments with respect to seed sowing rate was evident in the first harvest of biomass in both irrigated and non-irrigated growth. No differences were found between seed rate treatments in stem regrowth after defoliation. The stems of low seed rate under both irrigation treatments were more suitable for production of inflorescences per stem and for seed weight than high sowing seed rate.

Pulli (1980) reported that lucerne plants with taller stems under optimal temperatures showed early plant development than those with shorter stems. Among weather parameters, as reported by Brown and Tanner (1983), Martiniello (1998a) and Sinclair *et al.* (2006), the effect of water stress reduced the stems per plant in the early period of regrowth (first 14 days) and length of internodes in late stages of plant development.

The photosynthetic efficiency in stems and leaves is reached at different day/night temperatures. Composition of the structural carbohydrates in the stem mainly depends on temperature. In experiments established under controlled growth conditions by Smith (1970) on one-year-old plants of lucerne, red and alsike clovers, birdsfoot trefoil and perennial yellow sweetclover were grown in four temperature (°C) day/night regimes: 32/27, 27/21, 21/15 and 15/10. Results evidenced that flowering became delayed as temperature increased in birtdsfoot trefoil and biennial yellow sweetclover, plant and root dry matter increased as temperature decreased; tallest tillers were observed mainly in lucerne at 27/21. The number of tillers was not affected by the temperature regime, except in alsike clover. A positive correlation between the lignin to cellulose ratio in stems of lucerne and cocksfoot was found when the day temperature ranged between 21 and 32°C; temperature over this limit increased the lignifications of cell walls (Smith 1970).

Analogous results were found in field experiments by Sinclair and Hoire (1989), Zuoli *et al.* (2004), and Belesky *et al.* (2006) in prairie grass, perennial ryegrass, tall fescue and timothy. These authors collectively found that 27°C during the day and 18°C at night favoured highest biomass production. However, the photosynthetic activity of these species was reduced at night and during the day when the temperature was below 18°C and above 30°C, respectively. The growth of leaf and stem under day with 27°C growing condition was linearly related to leaf water potential and night temperature (Medrano *et al.* 2002; Kalaji and Pietkiewicz 2004).

The branching of the stem (relation between plant and shoot length) has been emphasized by many researchers whose experiments were carried out both under growth chambers and field evaluation. Studies made on lucerne indicated that the accelerated development of stems was associated with day/night temperature (Sato 1971; Pearson and Hunt 1972). In a field experiment established in lucerne by Martiniello *et al.* (1997) under a Mediterranean environment whose mean temperature of spring, summer and autumn (16, 32 and 22°C, respectively) favoured the development of tillers height (cm: 73 in spring, 79 in summer and 66 in autumn) and dry matter production (t ha^{-1}: 3.31, 2.37 and 1.81, respectively in spring, summer and autumn). In contrast, highest branching development and upright stem position was observed when the day temperature ranged from 20 to 28°C and the day/night temperature was 20/15°C for erect and 30/25°C for horizontal position. Pearson and Hunt (1972), Sato and Itoh (1974) and Wilhelm (2002), in lucerne grown in controlled conditions, observed that stem position depended to day/night temperature (day/night of 20/15°C favours erect position more than 30/25°C). Stem diameter development decreased as temperature increased. The association between the reduction in stem diameter and temperature was ascribed to lignifications of the stem by increasing the content of cellulose (Smith 1970; Bula 1972; Belesky *et al.* 2006).

Photoperiod influences stem length and development of the plant. An increase in photoperiod from 8 to 16 h favours stems height and diameter, and internodes length of the stem while stem weight is not affected (Sato 1971; Kalaji and Pietkiewicz 2004). In lucerne, timothy and white clover the photoperiod is correlated with stem elongation and temperature with plant development (Sato 1974; Suzuki *et al.* 1975; Volenec 1985; Todorovic *et al.* 1999; Zuoli *et al.* 2004; Long *et al.* 2006).

Solar radiation positively influences the number of stems per plant. Cowett and Sprague (1963), Sinclair and Muchow (1999) and Springer and Gillen (2007) reported that lucerne grown in the field under full sunlight produced more stems per plant than those growing under partially shaded conditions. The interaction between photoperiod, radiance and temperature interfere with the photosynthetic process of forage crop species. The metabolic routes of relocation of biochemical compounds in sink-source organs of plants determining the development stems is related to photosynthetic pathways used by forage crops to assimilate the CO_2 of the atmosphere, leaves and roots (Moss 1988; Medrano *et al.* 2002; Purcell *et al.* 2002; Kalaji and Pietkiewicz 2004; Volenec and Nelson 2007).

The physiological pathways adopted by plants to assimilate atmospheric CO_2 for producing about the 95% of the aerial Earth's biomass, is the photosynthetic process of C_3 plants (Raven and Edwards 2001; Blum 2004; Lawlor 2005; Wilhite *et al.* 2007; Ainsworth *et al.* 2008; Prabhakar and Rajib 2008; Mekuria *et al.* 2009). The basic metabolic process to fix CO_2 in all plants is photosynthesis of the C_3 plants described by Calvin and Bassham (1962) and well known as the Calvin cycle. The photosynthetic process under stress conditions (temperature over 30°C and water lower than 25% of those of soil water retention content), incorporate CO_2 as intermediate compounds by metabolic routes noted with C_4 and CAM (Crassulacean Acid Metabolism, i.e. resurrection or desert plants), plants and then assimilate by the pathways of C_3 plants. The CO_2 in C_3 plants is mediated by the enzyme Rubisco into two biochemical compounds with 3 atoms of carbons (3-phosphoglucerate). This reaction occurs in the mesophyl of leaves and represents the first step of the photosynthetic process.

The edaphic environments of C_3 plants require moderate sunlight and temperature, CO_2 concentration not lower than 200 ppm in the atmosphere and not lower than 30% of ground water retention content (Murphy 1986; Hobbie 2007; Pfanz *et al.* 2007; Jonathan and Bird 2008). A temperature over 30°C influences the activity of Rubisco which incorporates O_2 with an increase in temperature which favours photorespiration with a consequent reduction in the growth of plant organs (Erice *et al.* 2005; Martínez-Carrasco 2005; Xu and Zhou 2005; Martin *et al.* 2008; Ghannoum 2009). In dry areas, C_3 plants shut their stomata to reduce water loss and stop the entrance of CO_2, reducing its concentration in the leaves. C_3 plants utilize the stored biochemical compounds for increasing the photorespiration process to reduce the stress effect of weather conditions on organs (Hart *et al.* 1978; Volenec and Nelson 2007). The edaphic adaptation of biochemical mechanisms developed in C_4 and CAM plants fix CO_2 when the environmental weather conditions forbid the growth of C_3 plants. The pathways of photosynthetic routes of C_4 and CAM are alternative to photosynthesis of C_3 plants. The first step of C_4 photosynthesis pathways was discovered by Hatch and Slak (1966). CO_2 is assimilated by PEP-C in 3-acidphosphoenolpyruvic with four carbon atoms. The leaves of the first step of C_4 plants have a characteristics Kranz anatomy. C_4 leaves contain two incorporated cells types, thick-walled bundle sheath cells surrounded by thin-walled mesophyll cells. CO_2 enters in the upper mesophyll cells of leaves via stomata and is converted by carbonic anhydrase to bicarbonate acid. PEP-C oxygenase fixes bicarbonate acid into a four carbon compound OAA which is then converted to malic acid (malate) and shunted to bundle-sheath cells where it is split by malic enzyme to form pyruvate (3-C compound) and CO_2. CO_2 is fixed by Rubisco via the Calvin Benson pathway while pyruvate is shunted back to Kranz cells where it is converted back to PEP-C. The 4-carbon acids are transported from cell to cell through plasmadesma or pores from the mesophyll cells to the bundle-sheath cells where chloroplasts containing Rubisco is concentrated. However, the C_4 plants, in order to bypass the photorespiration pathway, have developed mechanisms of adaptability which efficiently deliver CO_2 to Rubisco in the Calvin cycle. Thus, C_4 plants have higher rates of photosynthesis than C_3 plants. Photosynthesis in C_4 plants is not saturated but increased at high light intensities and can continue at very low CO_2 concentration. Volenec and Nelson (2007) reported that the edaphic process of adaptation of C_4 plants came about due to warmer and dryer conditions during the period of plant evolution in Earth's history. The CO_2 concentration of the air in early period of Earth's formation was several fold higher than today so C_3 plants worked well but with an increase of light intensity and reduction of water availability to biochemical processes C_3 plants gradually shifted the metabolic process in those C_4 and CAM plants whose photosynthesis pathway was more efficiently released under limited and stressed weather conditions.

The relocation of biochemical compounds produced by photosynthesis in C_3 plants is lower than C_4 plants and as consequence the relocation of storage products in the organs of plants is substantially different between these groups of plants (Sage and Pearcy 1987; Jonathan *et al.* 2008; El-Sharkawy 2009; Ghannoum 2009). Perennial and annual cool-season C_3 accumulate fructans in stem bases, rhizomes and stolons, starch and sucrose in C_4 plants while C_3 plants of legume species store starch as carbohydrate in taproots, stolons and rhizomes. The leaf characteristics of forage plants with C_3 in comparison to C_4 plants showed a higher concentration in CO_2 in mesophyll cells [C_3 (40-100 ppm) vs C_4 (0-10 ppm)], leaf morphology [C_3 (palisade and spongy mesophyll, if bundle sheath present no chloroplast) vs C_4 (Kranz anatomy, with bundle sheath containing chloroplasts)], chloroplasts morphology [C_3 (1 type) vs C_4 (2 dimorphic types)], primary physiological fixation of CO_2 in mesophyll cells [C_3 (Rubisco) vs C_4 (PEP-C)], secondary physiological fixation of CO_2 in mesophyll cells [C_3 (none) vs C_4 (Rubisco separate in space bundle sheath)], primary CO_2 acceptor [C_3 (Rubisco) vs C_4 (PEP-C)], first stable product [C_3 3-PGA (3-PGA) vs C_4 (OAA)], transportation rate of photosynthate [C_3 (high) vs C_4 (about 25% lower than C_3)], optimum temperature for photosynthesis process [C_3 (25°C) vs C_4 (35°C)], concentration of CO_2 in mesophyll cells [C_3 (40-100 ppm) vs C_4 (0-10 ppm)], concentration of CO_2 in stomatal activity [C_3 (250-275 ppm) vs C_4 (175-225 ppm)], field photosynthetic rate per unit leaf area [C_3 (20-30 dry matter g m^{-2} d^{-1}) vs C_4 (40-50 day matter g m^{-2} d^{-1})], stomatal resistance [C_3 high (250-500 m^{-1} s) vs C_4 low (50-200 m^{-1} s)] and WUE (H_2O g for each g CO_2 gained [C_3 (400-500 g H_2O for CO_2 g) vs C_4 (250-300 g H_2O for CO_2 g)].

The photosynthetic process in both C_3 and C_4 plants happens during the day and the CO_2 present in the atmosphere in C_3 plants is directly assimilated in all mesophyll cells of leaves by Rubisco in biochemical compounds with 3 carbon atoms (3-phoshoglycerate) while in C_4 plants CO_2 is fixed in Kranz cells of mesophylls into compounds with four carbon atoms (OAA) and then shunted to the bundle-sheath where it is converted into a compound with three carbons atom by the Calvin cycle.

The CAM photosynthetic process is more evolved to adapt to harsh environmental conditions than that of C_3 and C_4 plants. Forage crops with CAM photosynthesis in Mediterranean environments are diffused in the Cactaceae (Indian fig). The flattened green branches (cladodes) of this species are used for forage. In field experiments in the Benghazi area of Libya (Jannelli 1975) and in two locations (Sardinia and Apulia) of Mediterranean regions of Italy with weather condition quite similar to those of a desert (Jannelli 1978), it was found that the biomass produced by Indian fig plants was successfully used for feeding mainly small ruminants all year round. The stomata in the leaves of CAM plants were closed during the day and opened at night.

CAM plants fix CO_2 at night by PEP-C kinase which catalyzes the formation of OAA which is transformed into malate by NADP and MDH enzymes. Malate is then transported into the vacuole where it is converted to malic acid which represents the storage form of CO_2 (Jenkins *et al.* 1987; Hong *et al.* 2008). The biochemical gap due to the loss of PEP-C activity as a consequence of high temperature and the concentration of malate, plants with a CAM photosynthetic pathway incorporate CO_2 at night and reduced the content of malate by transporting the compound into the vacuoles of mesophyll sponge cells (Ranson and Thomas 1960). Thus, CAM plants, during the day with closed stomata, photosynthesise using malate stored in the vacuole, which is transformed by PEP-C into a 3-PGA compound with three carbon atoms and CO_2. The released CO_2 in the mesophyll of leaves is then fixed into chloroplasts by Rubisco via the normal Calvin pathway while pyruvate is shunted back to mesophyll cells where is converted back to 3-phosphoenolpyruvic acid.

Thus, the CAM plants had sour testing leaves during the nights and sweet testing leaves during days. This is due, as reported by Raven *et al.* (2005) to the malic acid being stored in the vacuoles of the cells during nights, and its being used up during the day for Calvin cycle photosynthetic process which produced soluble carbohydrates.

In agreement with Hatch (1976), Hatch (2002) and Keeley and Rundel (2003), the C_3, C_4 and CAM photosynthetic processes pathway of external CO_2 is the consequence of a mechanism's adaptability to evolve under stressed weather conditions. The selective factor driving the evolution of the CO_2 pathway is excessively high photorespiration, which inhibits normal C_3 photosynthesis under high light intensity, temperature and water deficit during the day, providing a physiological mechanism for the Calvin cycle with CO_2 during the night as a 4-carbon atom compound. A well studied example reported by Björkman and Berry (1971) showed that *Atriplex* plants share both phylogenetic groups, i.e. spreading orache and red orache, which fix CO_2 using the C_3 and C_4 photosynthesis pathway, respectively. Open stomata only during the cooler and more humid night-time hours in CAM plants is a physiological peculiarity of photosynthesis, which permits the uptake of CO_2 with minimal water loss (Clanton and Osmond 2003; Wang 2008; Hong *et al.* 2008). Less than 30 botanical families with about 20,000 species have evolved CAM (Winteran and Smith 1996; Guralnick *et al.* 2008). Joy (2009) reported that the most common forage crops with the CAM photosynthesis pathway used for agronomic consumption are: pinto and rhizoma peanut, sunshine, sun hemp, Indian fig, lampuaye, kawelu, Mexican lilac, piligrass, dwarf Brazilian banana, paspalum, elephant grass, panax, akiaki, signalgrass, beach vitex and sleepy morning. Turfgrass and Kentucky grasses are forage crops used in molecular breeding programmes for introducing gene sequences of basal mechanisms of the CAM photosynthetic pathway for improving drought tolerance from genes of primitive meadow spikemoss plants and smut grass species of resurrection plants (Cushman and Perryman 2007; Silvera *et al.* 2009). A project of the Department of Biochemistry and Molecular Biology of Nevada University (NEV00341: 2007-2011) based on "Resurrection plants: Novel genetic resources for stress tolerance crops" studies the regulation of photosynthetic processes, testing the function of genes from two vascular desiccation-tolerant resurrection species (spreading Club Moss to the monocotyledonous angiosperm African Inselberg grass). The transfer of complex-tolerant drought genes to mouse-ear cress plant favours the development and establishment of gene sequences that can ultimately improve drought tolerance to non-drought-tolerant crops (e.g., turfgrasses and Kentucky blue grass). The aim of the Nevada University project in regulating photosynthetic processes is to establish a functional testing program for candidate genes with potential agricultural interest for conferring improved drought tolerance in cultivated crops. Thus, genetic engineering to manipulate the use of photosynthetic pathways of CAM plants for introducing CAM-related genes into C_3 and C_4 plants aims to reduce the negative environmental impact of agronomic crops (Silvera *et al.* 2009; Sweetman *et al.* 2009).

Leaves

The botanical, morphological, physiological and chemical characteristics of leaves are linked to the species of forage crops. In berseem, lucerne, cocksfoot and prairie grass moisture stress during plant development reduced mainly the area, size, weight of leaf and relocation process of stored N and carbohydrates in leaves, stems and roots. Evidence from field experiments showed that in annual berseem, the phenological stage of plant defoliation was the agronomical factor that influenced dry matter partitioning in stored compound organs of plants, namely roots, leaves and stems (Iannucci *et al.* 1996; Martiniello *et al.* 1996) while in perennial crops such as a legume (lucerne) (Martiniello and D'Emilio 1996; Justes *et al.* 2002) and grasses (cocksfoot, perennial ryegrass, prairie grass and smooth bromograss), the interval of shoot removal, based on leaf developmental stage of regrowth, affect dry matter and biochemical compound production partitioning in the leaves, roots and stems across the year of evaluation (Turner *et al.* 2006b, 2007). In perennial grasses, dry matter partitioning and water-soluble carbohydrates in organs of the plant, tiller regrowth, and number of tillers per plant were significantly affected by the phenological stage of the tiller at the stage when plants were defoliated. Results from Turner *et al.* (2006b, 2007) reported that the time of regrowth removal at an early stage of tiller development (lower expanded leaf), in lucerne and perennial grasses, produced less dry matter in the plant organs and stored fewer storage compounds than defoliation made on late tiller development with more expanded leaves. Particularly in perennial grasses, the defoliation interval had no effect on tiller number during the first year. However, in the following years of evaluation, tiller removal with fewer expanded leaves favoured the detriment of dry matter, plant density and the content of water-soluble carbohydrate in the plant's organs. Particularly in the Mediterranean environment, the development of lucerne is affected by the time of removal of regrowth and moisture content of the soil (Martiniello and D'Emilio 1997; Martiniello *et al.* 1997). Furthermore, in field experiments involving lucerne, Brown and Tanner (1983) found that water potential in field soil influenced leaf expansion and stem elongation. The growth of leaves and internodes results in nearly a constant rate during the vegetative regrowth under well watered conditions (water potential near 0 bar), and when water potential was -0.35 bars (30 cm from the Ap horizon) the growth of leaves and stems was reduced and the rate leaf over stem increased in comparison to the well-watered condition. Development of regrowth after 2 weeks under water stress (water potential = -10 bars), in comparison to the well-watered condition, reduced the leaf size, internodes length and dry matter production by about 50% while the number and length of internodes and stem density were not influenced. Thus, because leaves are more exposed than other organs to edaphic and to agronomic factors, the physiological processes which take place in them, i.e., photosynthesis and relocation of photosynthates, are consequently strongly influenced. Thus, in annual (berseem and Persian clovers and ryegrass of Italy) and perennial forage crops (red clover, perennial ryegrass, tall fescue, sainfoin and sulla) a reduction in soil moisture and increase in temperature affect the development of stems and root systems and stress and alter the quality of proteins, reduce the relocation of water-soluble carbohydrate in all organs of plants and inhibited nodule N-fixation in root systems (Iannucci *et al.* 2002; Jiang and Huang 2002; Aranjuelo *et al.* 2007).

In lucerne, as reported by Brown and Tanner (1983), and Martiniello and D'Emilio (1997), the effect of moisture stress which determine a reduction of 2 bars of leaf water potential, in comparison to well-watered leaves, decreased the area of total leaf size expansion per stem extension, the number of leaflets per leaf; the amount of leaf surface reduction that occurred during the period of water stress was related to the stage of stem development during vegetative growth (**Figs. 3, 4**).

Regression analysis in a study conducted in lucerne by Onstad and Fick (1983) under controlled temperature for predicting leaf stem proportion showed that an increase of 5°C base temperature on established stands with canopies ranging from 7 to 66 days reduced the weight of leaves more than stems in the standing herbage canopy (r^2 values of weight leaf: stem proportion ranged from 0.81 to 0.87). In lucerne, when leaves of 22-days-old plants were applied a controlled temperature of 20, 25, 30 and 35°C, the highest plant growth and leaf area were obtained at 25°C and lowest at 35°C (Bula 1972). Furthermore, leaves of plants treated at 20 or 25°C, when compared with those exposed to 35°C, had larger cells, more intercellular spaces, fully expanded leaves, thicker leaves and greater variation of leaf shape and specific weight (ratio between weight and volume).

The responses of specific leaf weight to temperature changes (higher, lower or unchanged weight/volume ratio) observed in lucerne were due to physiological process of starch accumulation whose metabolic pathway was reduced as the temperature increased (Bula 1972; Pearson and Hunt 1972).

Photosynthetic accumulation in environments with a day/night range of 20/15°C was about equal in stems without defoliation and faster in leaves of regrowth stems after defoliation. The different behaviours of berseem and lucerne leaves may be due to physiological processes caused by the accumulation sugars (glucose, fructose and sucrose), fructan and starch (Pearson and Hunt 1972; Martiniello *et al.* 1993a; Iannucci *et al.* 1996). In glasshouse experiment established with berseem, the amount (% over dry matter) of crude protein, total available carbohydrates expressed as percentage of glucose equivalent of dry matter and reduced sugars was related to the phenological stages of stem development (stages I and II: stems at the 4th and 8th internodes of elongation; stages III and IV: stems with 5% flowers and with seeds at physiological maturity, respectively). In berseem, the effect of tiller biomass removal based on phenological stage influenced the values of crude protein and carbohydrates partitioning in the organs of plant are summarized in **Table 1** (Iannucci *et al.* 1996).

The content of crude protein in leaves was highest in all stages of plant development in leaves more than in stems and roots. The higher CP content in leaves rather than other organs evidenced that the pathway of biochemical compounds production and their partitioning in stems and roots are regulated by leaf physiological activity. The decrease in CP content in all organs of phenological stage IV evidenced a decrease of physiological activity with an increase of phenological development of the plant. Furthermore, because the protein content was reduction from 31 to 19.7% in leaves, 27.3 to 11.3% in stems and 14.8 to 8.8% in roots, this was evidence that the physiological activities in the organs of plants are quite different. However, the higher value of GL in stems of phenological stage II resulted from a higher photosynthetic activity when glucose was temporary stored in the stem.

The stems and roots had a lower crude protein content in all stages of plant development, a consequence of the reduced physiological activity of the organs, 16.6 and 14.9%, respectively in stage I while in stage IV the content was also reduced (9.8% in stems and 10.2% in roots). The reduced RS and GL, except for the higher value of GL in phenological stage II of plant development, was 4.8 and 5.8%, respectively in stage I and remained around the same values at all stage of development. The little variation in RS in the organs across all phenological stages was evidence of reduced physiological activity of the compounds involved in the metabolic process of plant development.

Photoperiod influences the structure (morphology of mesophyll and palisade cells) and development of leaves. White clover, cocksfoot and timothy plants growing under a short photoperiod (9 h) showed slightly wider and longer leaf blades (in timothy: 6.8 and 154 mm, respectively) than those exposed to longer photoperiods (18 h) (leaf width and blade length in timothy: 8.2 and 282.3 mm, respectively) and as a consequence the specific leaf weight and leaf size depended on the amount of exposure to light during the growing season (Todorovic *et al.* 1999; Wu *et al.* 2004; Jackson 2009). A study carried out by Todorovic *et al.* (1999) on white clover shoots, by Sinclair *et al.* (2001) on subtropical forage crops (bermudagrass and bahiagrass) and by Wu *et al.* (2004) in timothy grass evaluated the influence of photoperiod on morphological and physiological parameters of plants during growth. Experimental timothy grasses grown in a climatic chamber exposed to 6-, 9-, 18-, 21- and 24-h photoperiods were determined, starting from the 5-6-leaf stage of development; the parameters measured were leaf tips, plant size and dry weights at 37, 46, 62 and 70 days of plant development. The authors found that plants responded to photoperiod, represented as a mathematical model. The developed algorithm described with a high level of precision ($r^2 > 0.97$) the effect of photoperiod on growth as expressed by leaf area development and aerial and root dry matter production. Furthermore, leaf area was more sensitive to photoperiod than the above-ground dry matter production. The results reported by Solhaug (1991), Wu *et al.* (2004), Claser *et al.* (2004) and Volair *et al.* (2009), respectively in cocksfoot, timothy, swichgrass and perennial temperate forage grasses (bulbosus barley and bluegrass, cocksfoot, harding grass and tall fescue) evidenced a functional mechanism for plant daylength which influenced the synthesis of biological compounds and their repartitioning in the organs of plants endowed with species-specific tolerance adaptability and responses to edaphic latitude. The selective advantage of a plant to photoperiod was the acquisition of a physiological mechanism which enabled the crop to establish the effect of photoperiod/day-night exposure on partitioning of pulse-labelled radioactive $^{14}CO_2$ in plant soil system of white clover (Torodovic *et al.* 1999). The authors determined that 24-h photoperiodic exposure did not significantly influence the radioactive $^{14}CO_2$ partitioning in leaves and stolons. A comparison of exposure to PTL and PTH evidenced that exposure to the latter favoured higher assimilation of radioactive $^{14}CO_2$ in leaves at the expense of organs such as stolons and roots. This was supported by a significantly higher activity of leaves compared to the roots. The reduction of $^{14}CO_2$ transferred to roots under low PTL was accompanied by a reduction of $^{14}CO_2$ found in the rhizosphere CO_2 and soil residues. This indicated that rhizodeposition of recently fixed C is correlated to C assimilation in the root. However, the fixed C relationship of root with microbial biomass activity is related to forage crops species and the quality of organic compounds released from roots (Bazot *et al.* 2006; Cheng 2008).

Mobilization of photosynthate in the vegetative organs

Plants, following defoliation, utilize stored compounds in the root system for rebuilding the photosynthetic activity in the organs of meristems (Fischer and Turner 1978). The period of time required for recovering autotrophic activity depends on the amount of compounds previously stored in the organ of a plant before defoliation and on soil and environmental resources (water, temperature and inorganic elements). The period for recovering photosyntetic activity is short when weather factors and stored compounds are available in optimal amounts; but if one of them is limited, physiological processes delay the rebuilding activity of the organs with a deleterious consequence on plant development. The physiological activity of the stored compounds after defoliation in roots, apical meristems and regrowth stems are, in comparison to the stems of previous defoliation, always reduced (Turner and Pollock 1998; Kertulis 2001; Turner *et al.* 2006b). The stored compounds present in above-ground and in active meristems are higher than those present in roots; thus under unfavourable soil water conditions, the plant is able to quickly recover the stress imposed by defoliation. The strength starvation of the sink organs is maintained until photosynthetic activity is able to satisfy the development of the regrowth stem. The roots and the active axillary meristems of the regrowth plant, when photosynthetic activity has been rebuilt, become sink organs for carbon and protein accumulation (Lemaire *et al.* 1992; Kim *et al.* 1993; Erice *et al.* 2006; Lestienne *et al.* 2006). In experiments by Meuriot *et al.* (2005), and Erice *et al.* (2006) in lucerne and Lestienne *et al.* (2006) in cocksfoot the impact of defoliation intensity and frequency with N supply on the N uptake, N mobilization and N allocation to roots, adult leaves and growing leaves was evaluated. On plants grown under controlled environments two rates of ^{15}N labelling were applied. In the experiments related to cocksfoot, the plants were reduced the percentage of leaf area by 0, 25, 50, 75 and 100%. These treatments were applied to a set of plants not defoliated and to a set defoliated several times at constant height. A ^{15}N tracer technique was used to quantify N uptake, mobilization and allocation over a period of 7 days. A significant reduction in plant N uptake was observed with the removal of more than 75% of leaf area only in high N supply. As defoliation intensity increase the amount of N taken up ad subsequently allocated to growing leaves during labelling period was maintained at expense of N allocation to root and adult leaves. The increase of defoliation intensity increased the relative contribution of root supplying mobilized N to growing leaves and decreased the relative contribution in adult leaves. Defoliation frequency not substantially alter N uptake, mobilization and allocation between roots, adult and growing leaves on a plant basis. The number of tillers per plant increased with repeated defoliation (equivalent to defoliation frequency), indicating that allocation and mobilization of N to growing leaves is based on the number of individual tillers. Because the physiological dynamics of N uptake described in cocksfoot was similar to those reported in lucerne by Meuriot *et al.* (2005); Kim *et al.* (1993) and Erice *et al.* (2006) it is possible to conclude the uptake of N mobilized and allocated in stored organs (root system and tillers) they were indeed mobilized to growing leaves after defoliation.

Plants with C_3 accumulated carbon biochemical compounds such as fructan while those with C_4 and CAM stored starch as a principal reserve of carbohydrates and soluble amino acid stored in the specialized organs located near or below the soil surface (Keel and Rundel 2003; Volenec *et al.* 2007). The breakdown and synthesis of starch grains during regrowth in C_3, C_4 and CAM plants are spatially separated in the taproot: grains near the vascular cambium are used before those located in the centre of the taproot (Hadden and Volenec 1990; Meuriot *et al.* 2003, 2004; Volenec *et al.* 2007).

The metabolic activity of starch utilization during regrowth is regulated by photoperiod. This finding was discovered by Meuriot *et al.* (2003) in lucerne, Wu *et al.* (2004) in timothy and Kallenbach *et al.* (1995) in sainfoin. These authors reported that metabolism of carbohydrate and protein in the regrowth of stems after defoliation is regulated by daylength while total non-structural carbohydrates and enzyme activity were not affected by photoperiod. The physiological process reported for legumes are basically similar to those of grasses. The difference between the two groups of plants (legumes and grasses) was found in the duration of the process. Pru'homme *et al.* (1993) and Lestienne *et al.* (2006) demonstrated that perennial ryegrass remobilised carbohydrate and soluble proteins from the stubble and roots to leaf regrowth during the first 6 days. After this period carbohydrates and proteins used for sustaining regrowth replenished the content of carbohydrates and proteins present before defoliation. During regrowth, and after defoliation of lucerne and white clover, the biochemical pattern of nitrogenase activity declined whereas the activity of β-amylase increased and shoot growth was replenished (Kim *et al.* 1991; Gana *et al.* 1998; Hogh-Jensen *et al.* 2002).

The active remobilisation of water-soluble carbohydrates stored in the reserve organs, as reported by Sinclair *et al.* (2006) in prairie grass, perennial ryegrass and tall fescue and Turner *et al.* (2006a, 2006b) in cocksfoot, are evidenced only during regrowth and not verified in non-defoliated plants. Relative to cocksfoot, Turner *et al.* (2006b) investigated the influence of leaf stage-based defoliation interval on water-soluble carbohydrate and N energy reserve status in regrowth leaves, roots and tiller numbers. The experiments were established under controlled conditions applying defoliation interval when the regrowth had 1-, 2- and 4-leaf stages. More frequent defoliation reduced water-soluble carbohydrate assimilation and therefore leaf, root, tiller numbers and dry matter accumulation during the subsequent recovery period. Defoliation at the 1-leaf stage severely limited the regrowth potential of cocksfoot plants, whereas defoliation at the 2-leaf stage was adequate for plant recovery, but did not maximize regrowth while a defoliation interval based on the 4-leaf stage maximized water-soluble carbohydrate in leaves, tillers, roots and dry matter yield. The priority of the allocation of water-soluble carbohydrate reserve during the regrowth period was in the order leaf, root and tiller. The N energy reserves were found to play a minor role in regrowth following defoliation.

The importance of N uptake on defoliation intensity on perennial ryegrass, Kentucky bluegrass, white clover and lucerne in regrowth has been questioned by Kertulis (2001), Meuriot *el al.* (2003) and Lestienne *et al.* (2006). These authors observed that the rate between defoliation and stem extension during regrowth in grasses (perennial ryegrass and Kentucky bluegrass) and legumes (lucerne and white clover) was not directly related to the carbohydrate content in the roots but to the content of vegetative-soluble protein in taproots.

In experiments carried out by Lasseur *et al.* (2007) under a controlled environment two varieties of perennial ryegrass with contrasting carbohydrate metabolism were evaluated. The plants were subjected to severe, frequent and infrequent defoliations and regrowth stress condition. Plants which had a greater content of fructans in leaf sheaths when subjected to defoliation treatments produced more leaf sheath biomass in regrowth plants than those with lower fructans. The contents of enzymes involved in the synthesising fructans (sucrose: sucrose 1-fructosyltransferase and fructan: fructan 6G-fructosyltransferase) declined after defoliation. Because the levels of gene transcription activity and regulation of expression did not decline concomitantly, the authors retained that the biochemical pathway of gene expression depends on the sink-source status of the carbohydrate in the leaf sheath tissue after defoliation; furthermore, the activity of enzymes during the phase of reserve accumulation increased more frequently than infrequently defoliated plants. Using this evidence as a starting point in cocksfoot, Turner *et al.* (2006b) and Sanada *et al.* (2007) retained that starch reserves in the roots are not directly involved in stem extension during regrowth. The active meristems remaining after defoliation, as found by Richards and Caldwell (1985) in slender wheatgrass and Meuriot *et al.* (2003) in lucerne, are more important during regrowth than the level of carbohydrate reserves in the crown. Stored carbohydrate in the roots and meristems played an important role in regrowth after defoliation. Some experiments reported that regrowth under stress conditions was positively correlated with carbohydrate pools and that regrowth was enhanced only when active meristems were available (Meuriot *et al.* 2003; Maleux and Van den Ende 2007).

Nitrogen compounds play an important role as the carbohydrate source during regrowth after defoliation. Kertulis (2001), Hogh-Jensen *et al.* (2002), Meuriot *et al.* (2003), and Lestienne *et al.* (2005) reported that plant respiration utilizes, in order to support shoot regrowth in the first 8 days after defoliation, most of the carbon and one-fourth of N reserve stored in the roots. In lucerne and white clover, the concentration of vegetative-soluble proteins, mainly asparagine and arginine, both amino acids, and three polypeptides with 32, 19 and 15 kD molecular mass – specific N compounds used for regrowth after defoliation – were determined by Hendershot and Volenec (1993), Corre *et al.* (1996), Avice *et al.* (2003) and Meuriot *et al.* (2004). Aspartate and asparagine were the most prevalent amino acids present in taproots and the concentration of these compounds with buffer-soluble proteins decreased greatly after defoliation (Kim *et al.* 1993; Noquet *et al.* 2001; Avice *et al.* 2003). In an experiment under a controlled environment, Lestein *et al.* (2006) placed perennial ryegrass under two treatments of N uptake and 5 treatments with different defoliation intensities (0, 25, 50, 75 and 100% of the leaf area was removed). In the plants after 7 days of defoliation, the N content (mg^{-1} plant) ranged from 124.1 to 12.6 in leaf area of 0 and 100% reduction and 2.9 to 2.2% under high and low N treatment, respectively.

These amino acids were postulated to serve as readily available forms of N whereas proteins may be long-term storage compounds. Ourry *et al.* (1994) and Volenec *et al.* (1996), using a controlled nutrient solution of N supply, found that, in lucerne, perennial ryegrass and white clover, the N compounds (vegetative-storage proteins) stored in roots and stem were degraded rapidly during shoot regrowth. Replenishment of the N reserves reaccumulated in the stored organs was made possible by photosynthetic processes when the leaves expanded (relocation from leaf to stored organs began 15 days after defoliation and continued until the defoliation).

In an experiment established with berseem clover genotypes subjected to defoliation treatments based on the phenological stage of plant development, the lower concentrations of N in the root (% of proteins/root dry weight) determined higher dry matter production of the stem during regrowth than the content of total available carbohydrates. The dry matter production of defoliation, made when shoots were at the 4-node stage of elongation, was 80.7 g and the content of protein and carbohydrates in the root was 13.8 and 5.3% of root dry weight. In contrast, when defoliation was performed during flowering of shoots, dry mater production was 94.1 g and the root dry weight was 10.5% for protein and 5.4% for total available sugar. The uptake of N by roots, as well as remobilization and dry weight during regrowth in legumes and grasses forage crops were more closely related to the amount of N (vegetative-storage proteins) stored in the organs of plants before defoliation than the water-soluble carbohydrates (Iannucci *et al.* 1996; Noquet *et al.* 2001; Meuriot *et al.* 2003; Turner *et al.* 2007). That was due to the fact that in agricultural ecosystems the time required for regrowth is related to quantities of water-soluble carbohydrate and vegetative-storage proteins in taproots. The amount of β-amylase activity and starch concentration were strongly paralleled by a decline after lucerne defoliation has higher β-amylase activity than other legumes (yellow sweetclover, red clover and birdsfoot trefoil). The reduction of stored N compounds in the roots was evidenced in experiments with lucerne plants grown in hydroponics culture with labelled ^{15}N. The study established in lucerne by Kim *et al.* (1991) was undertaken to define the sink and source behaviour of different organs in defoliated and intact plants. The results, over a period of 24 days of regrowth, evidenced that the N-remobilization used for regrowing in the first 10 days came from an endogenous source. Furthermore, 25-35% of stored N-reserves were translocated to regrowing stems. These observations suggest that the reduction of vegetative storage proteins and water soluble carbohydrates verified in the first 10 days after defoliation was used for sustaining by biochemical pathway of the regrowing plants. However, in agreement with Volenec *et al.* (2007) and Turner *et al.* (2007) the role of starch reserves in maintaining active meristems needs more investigation to explain the effect of defoliation on stored compounds in the remaining physiologically active organs of plants. Lestienne *et al.* (2006) and Turner *et al.* (2006a) consider defoliation tolerance of plants (rapid regrowth after defoliation) an agronomic advantage for enhancing high density and development of plants, particularly under unfavourable environmental conditions. Investigations on regrowth after defoliation of forage crops species underline the importance of management to safeguard axillary buds, which, if damaged, would reduce regrowth and the number of stems.

REPRODUCTIVE ORGANS OF DEVELOPMENT

The phenological development of forage, in terms of morphological stage of reproductive organ on dominant shoots of the plant, begins with the induction of flowering and terminates at the physiological maturity of the seed (Chastain and Youngh 1998; Casler *et al.* 2004). Soil moisture content is one of the most important factors involved in the switch from vegetative to reproductive phase in plant development (Sato 1971; Loeppky and Coulman 2001). The varieties of forage crop species are genetically characterized by temperature requirement and day length photoperiod for passing from the vegetative to reproductive phase of growth (Wu *et al.* 2004; Begonia and Begonia 2007).

The effect of stress on the different phenological phases of seed development differently influence plant organs of and yield components of seed production (**Table 3**).

Endosperm cell differentiation

The lag phase extends its period for about 15 days after pollination. It is characterized by cell division, differentiation of tissues and a rapid increase in seed fresh weight by accumulating solutes from plant organs.

During the lag phase of seed filling, environmental temperature and soil moisture influence the storage capacity of seed (Thomson *et al.* 1997; Alzueta *et al.* 2001). At the end of the lag phase, the number of endosperm cells and sites for starch and protein synthesis are potentially determined in the seed (Rebolé *et al.* 2004).

Lack of water potential in this phase reduces seed sink potential, accumulates stored compounds and prematurely arrests the development of seed which remain incompletely filled (Blevins and Barker 2007).

In angiosperms, seed sink potential is genetically determined but final seed production is a function of assimilate supply and competition among sink sites for space and resources available in the environment during the early stages of seed development (Akbar *et al.* 2006).

The environmental conditions during cell division influence development, the filling and relocation of starch, fats and proteins in the cells of the endosperm, the accumulation rate and the duration of stored products (Rebolé *et al.* 2004; Akbar *et al.* 2006).

The development of seed in the lag phase is particularly regulated by abscisic acid (ABA) and cytokinins. The level of ABA in the embryo, when the sink potential is established, is higher than the other organs such as endosperm, pedicel, placenta and chalazae tissues. An increase of tem-

Table 3 Effect of irrigation on seed yield and its yield components in annual and perennial forage crops established in two years experiments evaluated under different condition of water supply in Mediterranean environment (Elaborated from: Martiniello 2001).

Species of forage crops	Seed yield [b]		Seeds stem[c]		1000-Seed weight[d]	
	Rainfed	Irrigated	Rainfed	Irrigated	Rainfed	Irrigated
Annual legumes						
Berseem clover	183 a	456 b	25 a	26 a	2.68 a	2.91 b
Crimson clover	110 a	121 b	35 a	45 b	2.84 a	2.95 a
Persian clover	295 a	732 b	16 a	19 a	1.52 a	1.88 b
Squarrosum clover	328 a	493 b	28 a	32 b	3.49 a	4.08 b
Perennial crops						
a) Legumes						
Lucerne	184 a	332 b	51 a	73 b	1.81 a	2.16 b
Sainfoin	261 a	1554 b	33 a	152 b	24.9 b	18.3 a
Sulla	411 a	1024 b	46 a	148 b	11.2 a	12.4 a
b) Graminaceous crops						
Cocksfoot	181 a	362 b	40 a	82 b	0.67 a	0.91 b
Perennial ryegrass	171 a	285 b	52 a	57 a	1.37 a	2.01 b
Tall fescue	158 a	300 b	37 a	38 a	1.54 a	2.02 b

[a] Means species of rainfed and irrigated condition with same letter do not differ at $P \geq 0.05$ according to Duncan's Multiple-Range Test.

[b] kg ha^{-1}

[c] n.

[d] g

perature and water potential inside the organ of maternal origin promotes an increase of ABA and a reduced duration of starch synthesis in endosperm cells (Blevins and Barker 2007). Jiang and Huang (2002) and McCann and Huang (2008) retained that the increase of ABA concentration in endosperm cells may serve to downregulate the kernel sink potential. In many cereal crops, cytokinin concentration is considered as a positive effect in establishing sink potential and seed size (Sharma-Natu and Childiyal 2005).

The effect of environmental stress (water deficit and high temperature) strongly reduces the concentration of cytokines in the endosperm (Saini *et al.* 1997; Farooq *et al.* 2009). However, Landi *et al.* (2009) and Xu *et al.* (2009) in maize found that the influence of weather condition on the amount and role of cytokines and relation with ABA in the establishment and maintenance of kernel sink potential is not well defined.

Initiation of flowering

The genetic mechanisms of flowering initiation are specific for each species and are influenced by photoperiod and environmental factors (Falcinelli and Martiniello 1998; Rosellini *et al.* 1998; Jensen *et al.* 2004). Environmental responses of genotypes are closely related to the ontogeny of species which match target environments in such a way as to ensure good adaptation to climatic conditions (Fox 1989; Lloveras *et al.* 2001; Iannucci *et al.* 2007). Therefore, a variety within a forage crop species is characterized by photoperiod for flowering induction (Wu *et al.* 2004; Iannucci *et al.* 2006). The photoperiod required for flower induction is influenced by temperature. Johnson and White (1997) reported that the period of light required for flowering induction increased or decreased by low (lower than 17°C) or high temperature (higher than 28°C). Because there is a strong interaction between environmental factors and genotype, the effect of diurnal temperature on photoperiod, for flowering induction, is difficult to ascertain. In an experiment carried out on annual bluegrass by Johnson and White (1997) and Iannucci *et al.* (2007) on sulla, sainfoin, pea, berseem clover, field bean, common and hairy vetch, it was discovered that photoperiod interacts with low temperature (12-18°C) for floral development and generates variability in flowering time among varieties (Wu *et al.* 2004). Generally, the photoperiod for flowering induction in forage crops varies from 9 to 14 h with a moderate diurnal temperature (27/17°C). However, high night temperatures and exposure to long days brings flowering forward (Johnson and White 1997; Wu *et al.* 2004).

The age of the plant at which flowering can be induced varies with flowering type. In white clover genotypes, the sensitivity to flowering occurred at the three-leaf stage whereas a comparable response was not obtained in the late flowering type until more than 12 leaves were present on the plant. Moreover, the photoperiod needed to induce flowering varies with the kind of flower, type of crop, age of plant and high night temperature (Thomas 1979; Bernier *et al.* 1993; Guenni *et al.* 2005).

Critical components of photoperiodic control of flowering include a specific spectral response, action of a circadian clock to gate the photoreception input and production of florigenic signals that are then translocated from leaves to the tiller apex where they evoke flowering (Mouradov *et al.* 2002; Corbesier and Coupland 2006; Colasanti and Coneva 2009). Leaves are the predominant organ of day-length perception for regulating flowering. The inductive signal transported from leaf to tiller apex which favours flowering is florigen. This term was coined by Chailakhyan (1937), induced flowering by long-day photoperiod (LD, 12 to 14 h) exposure. Under a short-day photoperiod (SD, 8 to 12 h) the florigen signal is not induced and the plant remains vegetative. The well known florigen is an mRNA protein of the flowering locus gene *T* in darnel grass (*LtFT*) and the gene locus *Constants* in ryegrass (*LtCO*) and gibberellins hormones classes (GAs) (Mouradov *et al.* 2002; King and Evans 2003; Huang *et al.* 2005; Corbesier and Coupland 2006).

Darnel grass and ryegrass plants were grown in 8-h SD photoperiods in sunlight-controlled environment cabinets under growing conditions described by Evans *et al.* (1990). Flowering was induced by exposure to single LD by a low photon flux density (10 μmol m^{-2} s^{-1}) by incandescent lamps for 8 h or longer extensions of the main 8-h daily SD light period (Evans *et al.* 1990). Under these experimental conditions, Martin *et al.* (2004) and King *et al.* (2006) found that flowering regulation was induced by biosynthetic GAs, *LtCO* in ryegrass and *LtFT* in darnel grass.

The authors showed that under LD photoperiod, flowering signals may be induced, coupled to time measurement and generation and transport of florigen signals to the tiller apex, GAs and flowering locus genes *LtFt* and *LtCO*. Leaves were the predominant organ of LD perception of floregin protein of the *LtFT* and *LtCO* genes and GA class of plant hormones for florally inductive signals transported from leaves to the tiller apex (King and Evans 2003; Martin *et al.* 2004; Huang *et al.* 2005).

In forage crops (annual or perennial) that have defoliated, flowering depends on both night and day temperature and on the number of days required for stem regrowth to reach the phenological phase. Harvest following the first cut, in forage annual or perennial polycut crops, depends on night and day temperature which influence stem regrowth to reach the phenological phase of harvesting. In field experiments carried out by Martiniello *et al.* (1996) on berseem, Martiniello and Ciola (1994) in sainfoin and sulla, Martiniello (1998) in cocksfoot, ryegrass and tall fescue and Martiniello (1999) in berseem, crimson, Persian and squarrosum clovers, the first defoliation was based on the flowering phenological phase of growth; the time required for the following harvests for resuming the flowering response of stems was related to the environmental conditions in which defoliation occurred. The period between two consecutive defoliations depends on the photoperiod and weather conditions at the time of plant growth; it is shortened when passing from winter to spring and summer (Thomas 1981; Martiniello *et al.* 1997; Sanabria *et al.* 2008; Martiniello 2009). Information on factors that induce flowering initiation is useful for planning crop cultivation for the seed industry.

Pollination

In forage crops, reproductive development is particularly sensitive to environmental factors during blooming (Crane and Walker 1986; Kremen and Ricketts 2000; Dewenter *et al.* 2005).

In grass and legume crops, because they are mainly open pollinated, the reproductive stage is particularly dependent on plant development and on environmental factors (Lorenzetti 1993, Wilocok and Neiland 2002; Wang *et al.* 2004).

The pollen developmental phase is particularly sensitive to water availability and to the temperature of the environment (Kremen and Ricketts 2000; Dewenter *et al.* 2005). The main consequences of stress conditions occurring during the formation of female and male gametes are: abnormal meiosis (increase in univalent, lagging chromosomes, noncongression of bivalents in metaphase and formation of micronuclei), which strongly reduce the potential activity of pollination (Lalonde *et al.* 1997; Rossellini *et al.* 1998; Wilcock and Neiland 2002). The pollen rendered sterile fail to accumulate and distribute starch in the anthers and inhibit the development of pollen grains (Bosch and Waser 1999; Adler and Irwin 2006).

In plants under field condition, the lack of gametes was correlated to the photosynthetic rate and translocation of photosynthate from leaves to supply sucrose and other sugars to the anthers and mother cells. In this process, soluble acid invertase and cell wall invertase are the main enzymes involved.

Chaivisuthangkura (1998) retained that cell wall invertase activity is the enzyme involved in establishing a metabolic sink by maintaining a steep sucrose concentration gradient between sucrose and sink organs in higher plants. Apoplastic invertase plays a key role in the kinetic property of regulation, relation of sugar membrane loading and unloading transport system in the phloem. In an experiment established by Chaivisuthangkura *et al.* (1994), it was found that invertase is involved in the regulation of phloem transport and the partitioning of photoassimilates of the various carbohydrates in the organs of plants. The authors studied in carrot and mouse-ear cress, both higher plants, the physiological role of invertase by cloning the whole length of complementary deoxyribonucleic acid (cDNA) encoding the inverstase cell wall behind tissue-specific and inducible promoters in both sense and antisense orientations. The activity of cell wall bound acid invertase in transgenic calli derived from carrot and mouse-ear cress was assessed using *trans*-stimulation of the hexose cotransporter system as a signal. The cell wall acid invertase activity is related to water status in phloem systems whose alterations reduced the apoplastic activity of the enzyme (Dorion *et al.* 1996; Genter *et al.* 1997; Lalonde *et al.* 1997; Wingler and Roitsch 2008; Vogel 2008).

The life of mature pollen and egg cells depends mainly to the available soil water, temperature and wind (Hanson and Hitz 1982; Jenner 1982; Dorion *et al.* 1996; Saini 1997). During mild stress, egg cells remain alive until the ovary ceases to grow; but if the stress persists the cells discontinue development, the activity of acid invertase enzyme ends and then the pollen aborts (Sheoran and Saini 1996; Bosch and Waser 1999; Adler and Irwin 2006).

Mature microspores and egg cells depend on nonlethal environmental stress during formation. In a molecular experiment by Feurstenberg *et al.* (2000) in tobacco on anther, it was determined that meiosis is marked by the production of tetrads which are held together by a specialized callose wall which is responsible for the production, physiological function and degradation of pollen development. The authors, using polymerase chain reaction, amplified and subsequently cloned the cDNA which was expressed and regenerated exclusively in the anther during meiosis in the free microspore stage of pollen development. The proteins encoded had 31% glycine content and contained a putative signal sequence. Alignment of the nucleotide and amino acid sequences showed high sequence similarly to those previously detected by Kalaitzis *et al.* (1999) in tomato. The high glycine content in tobacco and the similarity with tomato sequences suggests that the protein functions as a structural cell wall protein involved in pollen exine formation. A similar experiment established on a legume (*Lotus japonicus*) was made by Endo *et al.* (2002) with the aim of understanding the molecular mechanisms intrinsic to microsporogenesis. The authors fabricated a cDNA microarray from flower buds to isolate genes which are specifically expressed in immature and mature anthers. The results suggest that cDNA microarray technology is an effective tool for identification of novel reproductive organ-specific genes. In both experiments the authors retained that the molecular mechanisms of pollen wall formation were susceptible to water movement in the flower during pollen dehiscence. Environmental stress affects the accumulation and distribution of starch in the anthers with a consequent reduction and inhibition of intine development (Hanson and Hitz 1982; Jenner 1982; Dorion *et al.* 1996; Saini 1997; Scott *et al.* 2004; Panter and Dickman 2005; Kölliker *et al.* 2010).

The accumulation of starch pollen during pollen development is used to sustain physiological activity linked to pollen germination and pollen tube growth (Westgate and Boyer 1986; Genter *et al.* 1997; Wang *et al.* 2004). The period needed for the ovary to complete maturation did not exceed 6 days (Saini *et al.* 1984; Lalonde *et al.* 1997b; Moïse *et al.* 2005). So, within this period, egg cells have to be pollinated otherwise they abort (Genter *et al.* 1997; Bosch and Waser 1999).

Perennial and annual grasses and legumes, with few exceptions (annual lespedezas, various beans, vetches, peas and subterranean clover) are partially or totally open pollinated crops. In grass forage crops, where wind is the principal pollinating agent, blooming normally begins near the apex of the inflorescence and proceeds more or less regularly toward the base of the spike whereas legumes are pollinated by an explosive tripping mechanism trigged by visiting insects (Heyn 1988). Insects that visit flowers apply pressure to keel petals allowing the staminal column to contact the stigma with foreign pollen stacked on the abdomen of the visitor promoting pollination with alien pollen collected from other plants. The most important use of insects in the pollination of forage crops belongs to the *Apoidea* family with a social or solitary life habitus (Satta *et al.* 2000; Martiniello and Pinzauti 2006; Hanlin *et al.* 2007).

Fertilization and seed initiation

Water plant status, air humidity and temperature and hot wind are the weather factors that can drastically reduce the fertilization of an egg cell (Sahramaa and Jauhiainen 2003).

In an experiment established on rice and tall fescue under controlled environmental conditions, it was found that low plant moisture content reduced the number of dehiscent anthers, amount of pollen shed and germinability (Ekanayate *et al.* 1990; Wang *et al.* 2004).

In cultivated crops, particularly in maize, the evolution of the stigma and anther in allogamous and insect pollinated forage crops is differently affected by environmental factors, with a consequent lack of synchrony between male and female development and a reduction of stigma receptivity time (Strickler and Richards 1997; Westgate 1994; Wilcok and Neidland 2002). Thus, fertilization of the egg cells by germinated pollen may fail or may be reduced. The different sensitivity existing among female and male organs to water potential of the plant may promote desiccation of the microspore and egg mother cell and lack of pollen fertilization (Westgate and Boyer 1986; Wilcock and Neiland 2002).

Damage caused by desiccation in the reproductive phase is irreversible, even if normal conditions surrounding development are restored. This could be due to damage promoted by low water potential of flower organs which irreversibly disrupt the cells of reproductive organs with a consequent loss of the zygote in the ovary (Westgate and Boyer 1986, 1990; Nelson *et al.* 2006). Environmental stress (mainly water, temperature and hot wind) at anthesis affects pollen viability and the capacity of pollen fertilization less than stigma receptivity. So, in open pollinated crops, appropriate management of agronomic practices (irrigation or reduction of pollination period) favoured synchrony between stigma receptivity and pollination, reducing the period of risk of the environmental stress on fertilization of the egg cell and development of the zygote in the ovary of the megaspore mother cell (Westgate and Boyer 1986; Fairey and Lefkovitch 2001).

Seed production

The fertile ovules in the ovary depend on pollination with a consequent allocation of the zygote into the ovary. Settlement of the zygote into the megaspore mother cell is a critical aspect of seed production in forage crop species. In experiments carried out by Elgersma and Sniezko (1988), Marshall and Ludlam (1989) in perennial ryegrass and Herrman *et al.* (2008) in red clover, abortion during fertilization was higher than 50%. The reason for the low rate of unfertilized ovules may be ascribed to genetic load of the forage crops, which determines a higher frequency of deleterious allelic combination, incompatibility of pollen grains and abnormality of the metabolic process at the beginning of cell division in the embryos of new seed (Weins *et al.* 1987; Marshall and Ludlam 1989; Lorenzetti 1993; Boyer and Westgate 2004).

The high percentage of zygote abortion is mainly related to the relationship between the biological activity of embryo formation for sustaining the request of the zygote in the ovary with the available plant resources. The main factor responsible for seed abortion, in the early stage of embryo in the ovary, is reduction of leaf water potential, which reduces photosynthesis and leads to a reduction of carbohydrate reserves in stems (Simon 1996; Rossellini *et al.* 1998; Genter *et al.* 1997; Wilcock and Neiland 2002). Therefore, abortion at the beginning of zygote formation may be attributed to a reduction in the supply of carbohydrates during pollination (Lorenzetti 1993; Rossellini *et al.* 1998).

Seed development in the ovary is dependent on the supply of assimilate from current photosynthesis, although carbohydrates continue to accumulate in vegetative sinks (stems, leaves and roots). This information is lacking in forage crops. However, in maize (*Zea mays* L.), mild water stress during pollination causes the relocation of photosynthates from leaf and stem to the reproductive organ to be severely reduced in the photosynthetic process (Marshall and Ludlam 1989; Boyle *et al.* 1991; Setter and Parra 2010). This indicates that early seed development is dependent on the supply of assimilates from current photosynthesis which cannot be replaced by the remobilization of reserves stored in other sink organs. So that, seed establishment in the ovaries depends on the rate of transfer of assimilates to reproductive organs rather than on the concentration of carbohydrates in the plant (Finch-Savage and Leubner-Metzger 2006).

To support this conclusion, results are mainly available in maize. Zinselmeier *et al.* (1995a, 1995b), in experiments carried out under controlled environments, experimentally manipulated water deficit, and carbohydrates were infused into the stems in an amount able to replenish that lost by inhibition of photosynthesis during the flowering stage. Also in maize, Boyle *et al.* (1991) and Zinselmeier *et al.* (1995a) in combined field, field under 55% shade and growth chambers found that the application of sucrose at 2 μmol m^{-1} s^{-1} prevented the abortion of the ovary, which decreased the number of kernels ear^{-1} by 40-70% more than the control grown under stress condition, namely withholding water supply at the day of silk emergence. The supplemental sucrose sustained ovary growth and cell turgor maintenance by osmotic adjustment. Thus, maintenance of a high level of sucrose in the ovary from current photosynthesis is essential for preventing seed abortion at the beginning of zygote allocation in the ovary sac. Studies carried out in maize under controlled environments indicate that the capacity of an ovary to utilize assimilate is related to external factors which promote stress in the mother cell. Zinselmeier *et al.* (1995c) and Setter and Parra (2010), in maize grown under controlled environments, evidenced that the physiological activity of carbohydrates and ABA metabolism in the ovaries of flower plants under water stress was strongly inhibited and the ovary ceased to grow, promoting a reduction in the accumulation of sucrose and a decrease in the level of reducing sugars.

Development of the microspore mother cell is more sensitive than macrospore gametes to water deficit during meiosis. An experimental study carried out by Saini (1997) under environmentally controlled conditions in wheat and in a field experiment by Fuzinatto *et al.* (2008) in *Brachiaria* hybrid found that water deficit during microspore formation causes male sterility. Water stress arrested male gametophyte development generating metabolic lesions which led to failure of male gametophytes. Physiological studies on alterations caused by unfavourable growth conditions on ovaries and microspores, concentrating primarily on maize, wheat and rice, evidenced that the alterations observed in these species were remarkably similar in other species (Chapman 1997). In many agronomic forage crop species Lorenzetti (1993), Simon (1996), Rossellini *et al.* (1998) and Herrman *et al.* (2005) reported the percentage of ovules present in mature floret converted in seed was 8% in lucerne, 25% in red clover, 40% in birdsfoot trefoil, cocksfoot and perennial ryegrass and 50% in white clover and tall fescue. The authors evidenced that the abortion of the ovules present at the initial stage of growth could be caused by a metabolic block resulting from a decline in invertase activity as a probable consequence of environmental stress.

Seed growth and maturation

The phenological events comprising the period from zygote nesting to seed physiological maturity is divided into three phases: lag, grain-filling and accumulation of stored compounds.

1. Lag phase

The phase begins with the allocation of a zygote in the ovary switching the vegetative to reproductive cycle of the plant. The embryo, endosperm and seed coat development begin 2 or 3 days after pollination. Consistent enlargement of the endosperm's dimension is evident 8 days after the allocation of the zygote into the ovary. The post pollination period is mainly susceptible to water deficit causing a lack of photosynthates, blockaging the translocation of carbohydrates from stems and leaves to the embryo (Wiens *et al.* 1987; Marshall and Ludlam 1989; Lorenzetti 1993). Photosynthetic restriction caused by defoliation at flowering in lucerne caused, under natural conditions, increased pod abortion and reduced seed per pod (Genter *et al.* 1977). However, in maize, water stress during the lag phase (3-8 days after pollination) in the post-gametic phase interferes with the mechanisms of sucrose, starch and ABA accumulation in the ovary, thus reducing seed development. Final seed development is strictly related to the intensity and duration of water stress during the lag phase (Boyle *et al.* 1991; Zinselmeier *et al.* 1995c; Setter and Parra 2010). In experiments carried out on maize under controlled environments by Boyle *et al.* (1991), photosynthetic products needed for seed yield was dimished by drought stress imposed during the initial phase of kernel development. The physiological mechanisms underlying this response were determined by sucrose, glucose, starch and ABA accumulation in apical and basal kernels during water deficit. Maize plants were grown in a greenhouse in 20-L pots containing 22 kg of amended soil. Water was withheld on the first day silk emerged, and plant was hand pollinated 4 days later imposing a decrease in leaf and silk water potential (-1.8 and -1.0 MPa, respectively). Plants were rehydrated 2 days after pollination. The brief water deficit, compared to the control, inhibited ovary growth (dry matter accumulation) and decreased kernel number per ear by 60%. The inhibition of ovary growth was associated with a decrease in the content of reducing sugar, depletion of starch, increase of sucrose concentration and inhibition of invertase activity. Water deficits during pollination disrupted carbohydrate in the ovaries. The authors suggested that that acid invertase activity is important for establishing and maintaining reproductive sink strength during pollination and early kernel development.

2. Grain filling

During seed filling, the established potential sink sites begin to accumulate proteins and starch reserves. The duration of the filling phase ranges from the end of cell differentiation to physiological maturity of the seed (20-30 days). This period is under genetic control and the biological processes of relocation of products from plant sink organs (mainly stem and leaves) to seeds, are influenced by environmental conditions. Among them, the water potential of leaves and seed reduced the metabolic activity of the organs and alter the process of seed filling. Westgate (1994) for some cereals and Wang *et al.* (2008) in tufted vetch reported that the effect of environmental stress (moisture, temperature or wind) reduced the period and duration of seed filling; as a consequence, poor seed size and weight resulted. Therefore, the effect of environmental stress during seed filling causes premature cessation of metabolic activity and a lack of assimilate supply. The consequences of stress on grain filling are associated with leaf senescence and with a decrease in metabolic activity of the embryo and endosperm cells which promotes the premature desiccation of seed (Egli 1994; Lemke *et al.* 2003).

Another mechanism that favours dissectation in the development of seed is the water potential of embryo cells. During seed filling the water potential in maize, wheat and soybean remained strictly constant in the range -1.61 to -1.99 MPa in maize, -1.52 to -1.63 MPa in soybean while in wheat it remained strictly constant at -1.66 MPa. These water potential values, at physiological maturity of the embryo (maximum seed dry matter accumulation), increased across the species rapidly in the range of -2.07 to -2.20 MPa (Egli and Tukrony 1997). Thus, the status of water content in seed maturation is a trigger of cellular desiccation which limits the synthesis and relocation of storage reserves, even when assimilates are available (Adams and Rinnie 1980; Westgate 1994; Samarah *et al.* 2003). However, water stress in maize limits grain filling, thus decreasing final kernel development but does not alter the rate of carbohydrates and N compounds in dry matter (Westgate 1994).

The ABA content in the endosperm of maize increases from the early phase of development until physiological maturity of the endosperm when its concentration falls. The reduction in dry matter accumulation in late grain filling may be attributed to dehydration of the endosperm cells rather than a previous hormonal signal (Westgate and Boyer 1986; Westgate 1994). Walbot (1978) reported that the effect of ABA during the grain filling process regulates water relations and growth of the embryos by preventing an increase in hydrostatic pressure promoting solute accumulation in the endosperm.

Variations in endosperm water potential during grain filling were detected in experiments under a controlled environment by Egli and Tukrony (1997) in maize, wheat and soybean and Shackel and Turner (1998, 2000) in chickpea and faba bean, may be due to the consequence of osmoregulation of solute accumulation in the cells of the embryo and endosperm promoting desiccation of seed whose initial physiological and metabolic activities are not reversible even if cell turgor is restored by optimal growth conditions. The effect of desiccation favoured by abiotic stress, as reported in mouse-ear cress plant by Maurel and Chrispeels (2001), Ramanjulu and Bartels (2002) and Moore *et al.* (2008), induced protoplasmic water associated with the cell wall which determined the physiological and biochemical status at the cellular and molecular levels.

The recovery of the physiological activity of unstressed condition was determined by a complex of genes involved in the synthesis of channel proteins (defined by Ramanjulu and Bartels (2002) as zipper proteins) that control the specific transport of water across the cell wall membrane, and found that at least five signal transduction pathways control the activity of the genes: two are dependent on ABA, and the others are ABA-independent. In addition, a numbers of genes for protein kinesis and transduction factors may be involved in the stress signal by environmental stress which influenced the intrinsic hydraulic coefficient of cell wall; thus, the repair of the initial physiological activity never reached the previous aquaporins proteins (Marte *et al.* 2002; Ramanjulu and Bartels 2002).

Rehydration of the stress condition determined the incomplete recovery of intrinsic hydraulic coefficient which elicits profound cytological changes, namely vacuole fragmentation, thickness of the cell wall and the morphology of plastid and mitochondria which caused a significant reduction in relative water content and an increase in the ABA, GA and protein level in the peduncle (Marte *et al.* 2002; Wand *et al.* 2009; Prassel and Dukett 2010). In rice, Muthurajan *et al.* (2011) fount that the lack of recovery of the initial physiological activity when plants were rehydrated was due to ABA-GA antagonists in the synthesis of proteins which determine panicle extension under drought stress and consequent increase in spikelet sterility.

The effect of environmental stress during grain filling promotes cavitations of morphological vessels in the ovary which determine the hydraulic isolation of seeds from other structures of the plant. These variations in seed cell turgor involve vascular discontinuities and cavitations in the seed structure, xylem of the stem and osmotic regulation in the apoplast (Domoney *et al.* 2006).

Variation in tugor pressure of developing embryo cells of chickpea and faba bean in the seed coat was stable over time and was found to be -0.1 to -0.3 MPa for chickpea and -0.1 to -0.2 MPa in faba bean. In both species there was a marked decline in cell turgor when water in the ovary was discontinued (Shackel and Turner 1998; Ma *et al.* 2001). The loss of cell turgor in the absence of water availability in the ovary raises serious concerns regarding desiccation allowing reduction in seed water potential and a decrease of water in the endosperm. The reduction of water in seed induces alterations in the water status limiting the inflow of assimilates into the embryo and endosperm (Shackel and Turner 1998; Ma *et al.* 2001).

Egli and Tekrony (1997) in maize, wheat and soybean, Rossellini (2004) and Zhang and Wang (2007) in forage turf grass and forage crops found that cessation of cell expansion determines seed desiccation and the arrest of metabolic processes during seed maturation. The end of translocation of storage reserves to seed is stopped when the water potential in cells increased promoting dehydration during seed maturation. Therefore, seed water volume determines final seed size in storage reserve accumulation and establishes the duration of seed filling. Shackel and Turner (1998) in chickpea found that damage of vascularized tissues of maternal organs promoted by osmotic turgor stress in cells of the pod wall and seed coat was not entirely reversed when normal or optimal growth conditions were restored. Thus, when the cell turgor of the maternal tissues is altered the metabolic processes of accumulation cease (Westgate 1994; Egli and Tekrony 1977; Shackel and Turner 1998).

The metabolic mechanism which promotes water uptake and maximum seed water volume has not yet been established. In experimental conditions, Westgate (1994) and Egli and Tekrony (1997) measured in maize, wheat an soybean plants exposed to water deficit during grain filling, a decrease in assimilate supply to seed and an alteration of the osmotic potential gradient passing from -1.60 to -2.2 MPa. However, when the water potential reached -2.6 MPa the growth of endosperm ceased.

This finding is not well supported by evidence in forage crop plants. However, in the maize endosperm, maximum water content is reached during rapid seed filling, although the physiological mechanisms in support of the assimilation for rapid dry matter and solute accumulation to sustain the osmotic gradient of water flux have not been well investigated (Westgate 1994; Zhang and Wang 2007).

Thus, the physiological mechanisms involved in seed endosperm for achieving maximum water content in early grain filling still remain indiscernible. In maize, Shussler *et al.* (1984) and Westgate and Boyer (1986), and Schussler *et al.* (1984) in soybean under field and *in vitro* culture evidenced that the physiological mechanism of water in endosperm cells is governed by ABA activity which controls assimilate transport and storage of soluble sugars in the embryo and endosperm. In both experimental conditions, the concentration of ABA in the embryo increased rapidly during early to physiological maturity (maximum increase of dry matter) suggesting that it acts as a trigger rather than as factor to mediate grain filling. The moisture content and dry matter accumulation in the endosperm are insensitive to osmotic potential in the range of -1.1 to -2.2 MPa while an osmotic potential below -1.1 MPa was sufficient to trigger the loss of kernel water content, cell turgor, the content of osmotic potential water and induced synthesis of ABA in an amount able to support the growth of the kernel for only a few days.

The plant moisture content at the end of seed maturation in fructiferous organs of annual and perennial legumes and grasses species ranges from 40 to 45% (Elias and Copeland 2001; Najada 2005; Wang *et al.* 2008). Seed harvest is related to drying of morphological parts of plants until the seed reaches 10-12% moisture content. A reduction in plant moisture among seed growers is achieved by swathing, herbicide application and natural drying in the sun. The choice of preharvesting drying system is related to the environment. In the northern part of the world (Northern Europe, USA and Canada) swathes and spring herbicides are quite diffusedly used while in Mediterranean environments plants will be dried naturally by sunlight (Martiniello 1998a; Steiner *et al.* 2006). The pattern of water content during seed development varies considerably among forage crops. A large variation of the final reserve stored in the seed reflects differences in genetic potential as well as environmental effects on seed growth.

The accumulation of storage reserves replaces the seed water volume leading to tissue desiccation (Genter *et al.* 1997; Elias *et al.* 2001; Ma *et al.* 2001; King *et al.* 2006). The process of seed filling accumulation of stored compounds during seed development of forage crops is strictly linked to environmental conditions which influence endosperm and ovary cell turgor, water leaf potential and activity of ABA and invertase activity (Branner 1987; Shackel and Turner 1998; Jones and Abo-El-Kheir and Mekki 2007). The relationships between water content in the endosperm and embryo cells and seed accumulation of the stored compound implies a common physiological mechanism which may control the cessation of growth and the water status of the seed. Therefore, it is reasonable to assume that synthesis and the end-product of storage such as starch, protein and fat during seed-filling processes requires optimum coordination among substrate availability, enzyme activity and translocation of solutes in the permanent sink organs. Water stress on physiological activities during seed growing in rice (Yang *et al.* 2003), wheat (Yang *et al.* 2004) and maize (Setter and Parra 2010) enhanced sink activity of ABA, which plays a vital role in the regulation of the activity of key enzymes involved in sucrose to starch conversion (sucrose and soluble starch synthase, starch brancing and ADP pyrophosphorylase) during grain filling (Boyer and Westgate 2004).

In an experiment carried out in maize, rice and wheat grown under water deficiency and a well-watered control, the progressive loss of water from the endosperm and restriction of water uptake by the embryo led to a rapid decline of water potential in the organs with a decrease of ABA, ADPglocose pyrophosphorylase, sucrose and soluble starch synthesis, and the activity of starch branching enzymes. The loss of enzyme activity reflects a decreased capacity for protein and carbohydrate synthesis as water content declines. Therefore, it is possible that the decrease of endosperm water potential may be due to a weak concentration of solutes in the organ of seeds and in advanced maturity, due to water potential (Westgate 1994; Yang *et al.* 2003, 2004; Zhang and Wang 2007).

Fructiferous organs under low moisture content may shed or promote, as in the case of most pods of legume crops, suberization of the apoplast membrane of the seed peduncle. The consequence of both situations is a loss of seed from fructiferous organs (dehiscence in legumes) or seed fall from the head (drop of spikelets) in grasses. Scientific experiments on the physiological process of suberification of the abscission zone in the peduncle of the ovary in forage corps are scant. However, water stress applied to cotton and citrus grown in a phytotron experiment carried out by Pline *et al.* (2003) and Iglesias *et al.* (2007), reduced the levels of fructose, glucose, starch and PGRs (ABA, auxins, GAs, cytokinins and ethylene) in the boll and citrus promoting abscission and senescence of fructiferous organs.

The mechanism of dehiscence is genetically controlled (Abo-El-Moneim 1993; Mosjidis 2007). Because seed legume represent an important seed source mainly in vetch species for West Asian and North African regions, scientists at the ICARDA centre began a breeding program since the 1980s to develop higher-yielding varieties with non-shattering qualities. The improved varieties were used in a barley-vetch rotation management with fallow; yields improved and there was better use of water efficiency than an ancient barley monoculture (ICARDA 1998). However, breeding programs to reduce the effect of dehiscence in legume crops is not diffused across private and public laboratories or research institutes; thus, seed loss by shattering still remains troublesome in legume seed production.

The ventral sutures of the carpel margin open along the median vein of the pod shattering the seed out (Garcia-Diaz and Steiner 2000; Rossellini 2004). Nowadays, this phenomenon is still quite diffuse in forage crops with a long seed-vessel cylindrical pod (birdsfoot trefoil, fenugreek, faba bean, pea, common and tufted vetch, yellow sweetclover); the consequence is a tremendous loss of seeds during harvest (Jarso and Keneni 2006; Wang *et al.* 2008). The shatter begins when the seed dry weight reaches the maximum value. The effect of shattering during harvest in legumes crops with a seed-vessel pod has been studied by Wang *et al.* (2008) in tufted vetch species. Under field conditions, experimental flowers of tufted vetch were tagged at anthesis and after 9 days tagged pods were harvested at 3-day intervals beginning from day 9 and continuing until pod shatter. A gradual change was observed in pod and seeding colour

Table 4 Yearly **g**enetic gain (difference between experimental two years means values of released varieties and variety divided ten) of UE variety released in the last decade over tester variety for seed yield and yield components traits (unpublished data of two years experiment established in Mediterranean environment).

Species of forage crops	Variety evaluated [a]	Genetic gain in dry matter, seed yield and seed yield component traits in 10 years released variety					
		Dry matter yield [b]	Seed yield [b]	Harvest Index [c]	Heads stem [d]	Seeds head [e]	1000-seed weight [f]
Common vetch	6	9.2	0.1	0.2	0.1	1.1	5.8
Hairy vetch	5	1.8	-1.9	- 0.0	0.1	0.5	0.5
Berseem clover	13	2.1	0.7	0.3	0.3	0.5	-2.2
Persian clover	4	2.2	-7.3	-04	0.2	1.8	-2.1
Sainfoin	3	2.1	-0.9	0.9	0.1	0.4	3.2
Sulla	4	2.1	1.4	-0.3	0.9	2.1	2.4

[a] n.
[b] kg ha^{-1} year^{-1}
[c] %
[d] n.
[e] n.
[f] g

with a progressive development until pod shatter. In the first 9 to 15 days after pollination (DAP) the pod was light green, turning green from 18 to 27 DAP, brownish-green from 30 to 33 DAP and light brown from 36 to 42 DAP. The seeds inside the pod in an early period of development were transparent in the first 15 DAP, becoming green in the period from 18 to 27 DAP and greenish-black and then black and hard from 36 to 42 DAP. The seed at 36 DAP from anthesis reached maximum percentage germination and seed quality; at 42 DAP from anthesis the germinability of seed was reduced, as was seed quality and moisture and pods started to shatter. Thus, tufted vetch can be harvested 6 days before shattering without affecting yield. After 42 DAP, the loss of seed due to shattering depends on the variability in homogeneity existing among pods in the species to reach the shatter phenological stage. However, flowering and seed development among tillers range from 2 days to 2 weeks, resulting in seed ripening at different times. The loss of seed in tufted vetch was related to the number of totally ripe seed at harvest. In canola, Gesch *et al.* (2005) in a field experiment, harvested the crop at 15-day interval times and found that the loss in seed yield of late harvest in October increased from 30.1 kg ha^{-1} d^{-1} in the harvest in mid-August to 10.6 kg ha^{-1} d^{-1} in mid-October. The authors retained that the sharp decline in seed yield was a consequence of increased shattering. In a spray application, Baig *et al.* (2003) noted that in pea, when moisture of the seed had reached about 30%, seed was lost due to shattering while qualitative seed characteristics (germinability and seedling growth) also decreased. However, nowadays until shatter-resistant genotypes are developed, improved agronomic harvest management is needed to reduce shatter-induced seed yield loss.

An analogous problem related to seed loss from spikes in cereals grown for seed consumption has been drastically reduced by selection (Baenziger *et al.* 2006). Unfortunately, because forage crops are grown for herbage biomass consumption, breeding selection efforts were devoted to herbage production instead of seed yield. Thus, scientific approaches of breeding programs related to seed production are considered to be a secondary aspect in achieving genetic improvement of seed yield potential (Lorenzetti 1993; Simon 1996). To date, the genetic gain of breeding programs aimed at seed production of forage crops released in the last 10 years has been very low (**Table 4**). Thus, genetic efforts for improving the factors involved in seed industry production are still quite far from being solved. Therefore, a reduction in seed loss in cultivated varieties of both legume and grass crops may be achieved by adopting appropriate management strategies which consist of harvesting the crop when seed moisture content of the plant is near 25%. This level of humidity in tillers prevents seed shattering in pods and suberification of the calazal peduncle of seed in spikes reducing the phenomenon of natural seed loss (Elias and Copeland 2001; Najda 2005; Jarso and Keneni 2006). To preserve seed quality (seed germinability, vigor and extension of seed aging) the moisture content after threshing has to be suddenly reduced to 10% (Elias and Copeland 2001; Najada 2005; Stainer *et al.* 2006). Thus, harvested seed have to be rapidly dried using industrial driers until the seed moisture content reaches – in a short period – the humidity content which allows safe storability of seed in a warehouse without loss of seed quality until packaging (Elias and Copeland 2001; Eichelberger *et al.* 2001; Wang *et al.* 2004; Groot and de Groot 2008).

SEED PRODUCTION

Seed production in forage crops is a complex process that involves the relationship between a large number of biotic and abiotic stress factors and environmental conditions.

The number of mature ovules present at flowering is quite large compared to the amount of seed harvested. The potential number of eggs of mother cells is very high for all forage crops. Quesenberry *et al.* (2003) and Rossellini (2004) reported that the number of ovules that successfully evolved to seed was 8% for lucerne, 50% for red clover and tall fescue, 25% for white clover and 40% for perennial ryegrass, cocksfoot and birdsfoot trefoil. Selection programs in forage crop species for seed yield improvement have been not incisive enough to solve the problem of seed production (**Table 4**). Thus, an increase in the seed industry may be achieved through a careful choice of agricultural practices (Swain and Koltunow 2006; Martiniello 2008; Zhang *et al.* 2008).

Seed yield components

The components of yield, namely stems/unit area, fructiferous organs/stem, seeds/fructiferous organ and seed weight play a fundamental role in determining industry seed production. By dissecting seed yield into independent components provides a better opportunity to understand the relationships that exist among and between them. Yield components are under genetic control and are strongly influenced by environmental weather conditions during vegetative and reproductive plant development. The genetic gain of seed yield and its yield components, in forage crops, is very low (**Table 4**). The genetic variation of traits attributable to the additive genetic component is small in comparison to the phenotypic variation observed in environments (Martiniello and Iannucci 1998; Annicchiarico *et al.* 1999). Breeding programs used to improve seed yield in forage crops are scant (Claser *et al.* 2000; Barret *et al.* 2005; Baenziger *et al.* 2006). The selection process is used to exploit unknown specific genetic variability, to release adapted genotypes, and to adopt simplified genetic breeding methodology (Martiniello 1992a, 1992b; Martiniello *et al.* 1993; Barrett *et al.* 2005; Herrman *et al.* 2008).

Martiniello and Iannucci (1998) established a breeding experiment for improving forage and yield potential in a berseem population. Nine higher forage plants were selected from an agronomic evaluation of six populations used as raw breeding materials. The seed from selected plants were used to make a natural isolated crossing block. The seed of each plant was harvested and used as a source of half-sib family and an equal quantity of each of the nine plants was bulked to make seed of an improved family source. Seeds of 54 half-sib maternal plants, six advanced and as many natural populations were used for implanting agronomic field trials to determine forage and seed genetic parameters. The forage evaluation was assed applying on the collected populations two harvest treatments: a short cycle (defoliation made when the 7th-8th internodes elongated) and a long cycle (defoliation made at flowering) while the populations used for seed yield were never defoliated. The traits investigated were: genetic variability, narrow sense heritability, genetic and phenotypic correlations among forage and seed yield and seed yield component traits. The study evidenced that genetic variability and narrow sense heritability among the half-sib family was higher by 51 and 35% in the short rather than the long cycle while narrow sense heritability in seed yield was 35% lower than seed weight. The magnitude of genetic variance components and genetic correlations suggested that selection among plants of maternal half-sib populations would be more effective for improving dry matter in a short than in a long cycle of defoliation. Selection applied in the study was not effective for increasing seed yield which may be increased by selecting indirectly for a seed weight trait.

The assistance of physiological approaches in seed yield selection is useful to better understand the relationships between traits and to discover the genetic interactions with the environment (Iannucci *et al.* 2000b; Turner *et al.* 2000). Alves de Araújo and Coulman (2004) and Martiniello (2005) proposed, in forage crops, to associate the breeding methodology with indirect morphological and physiological parameters that contribute to improve selection efficacy mainly for seed yield. Stanhill (1987) and Kelman and Ayres (2004) affirmed that seed yield is related to the amount of water transpired by crops and to efficient physiological metabolism which promotes the exploitation of environmental resources in phytomass and in seed.

The relationships among physiological, biochemical and morphological traits represent an integrated function of seed yield production which may be evaluated considering the correlation between parameters among seed yield and its yield components (Harwick 1988; Martiniello 1998a, 1998b; Iannucci *et al.* 2000b; Alves de Araújo and Coulman 2004). The response of genetic improvement achieved in annual clovers (berseem, crimson, Persian and squarrosum), perennial (elephant grass, lucerne and white clover) forage crops in environments with Mediterranean and tropical climates to seed yield and seed yield components has been determined by correlation and path analysis (Iannucci and Martiniello 1996; Annicchiarico *et al.* 1999; Sengul 2006; Zhang *et al.* 2010).

Studies on seed yield which consider seed yield components and the physiological effect of water and temperature during grain filling are useful for acquiring indispensable knowledge for developing genotypes with physiological attributes able to better utilize environmental resources. Information on the relationships among physiological mechanisms involved in seed yield components provides a scientific tool able to analyze genetic variability and WUE associated with superior yielding genotypes under limited water conditions (Stanhill 1987; Wright *et al.* 1996; Martiniello 2005).

Forage crops grown in a Mediterranean climate can lose up to 60% of available water by soil evapotranspiration (Loss *et al.* 1997; Martiniello 1998b). In environments with a typical Mediterranean climate, defoliation management strategies cover an important agronomic role for seed production in forage crops. Plant regrowth after defoliation has to reach development able to guaranty a canopy which reduces soil water evaporation. The number of defoliations before seed yield production generally is one; however, this depends on water availability present in the soil and on the ability of a crop to regrow a plant with a canopy able to reduce soil evaporation.

Table 5 Mean and their standard error, of seed yield and seed yield components traits over 4 clover species and mean over a 6 genotypes of berseem clover under different weather and defoliation condition of growing in Mediterranean site (Elaborated from: Martiniello 1999 four clover species, and six berseem clovers genotypes by Martiniello and Ciola 1995).

Trait	**Water available (± SEM)**		**Rainfed (± SEM)**	
	Never defoliated	**Defoliated**	**Never defoliated**	**Defoliated**
4 clover species[£]				
Seed yield [a]	451 ± 35	142 ± 7	228 ± 41	47 ± 12
6 berseem clover genotypes[$]				
Seed yield [a]	440 ± 34	210 ± 29	190 ± 24	105 ± 32
Heads stem^{-1} [b]	43 ± 3	36 ± 3	30 ± 3	25 ± 5
Seeds head^{-1} [c]	19 ± 1	17 ± 1	17 ± 1	15 ± 1
1000 seed weight [d]	2.55 ± 0.05	2.40 ± 0.05	2.31 ± 0.05	2.20 ± 0.04
HI [e]	14 ± 2	21 ± 2	25 ± 2	25 ± 2

[£] Mean over four years and four species of clover: berseem, Persian, squarrosum and crimson evaluated in experiments in Mediterranean environments.

[$] Mean over two years of 6 genotypes of berseem clovers: Alex, Axi, Lilibeo, Sacromonte, Ecotypes chietino and toscano, evaluated for two years under a Mediterranean environment.

[a] kg ha^{-1}

[b] n.

[c] n.

[d] g

[e] %

The metabolic routes of seed yield and its components are influenced by soil water availability and photosynthates present in the organs of a plant during the transition phase of development. In field experiments made on annual and perennial forage crops, seed yield was reduced as well as modified water conduction and defoliation of stems before early flowering in four species of clovers, six genotypes of berseem (**Table 5**) and three perennial grasses and legumes (**Table 6**) (Martinillo *et al.* 1996; Martiniello 1998, 1999).

Seed yield experiments carried out by Martiniello (1999) on the most diffused genotypes of four clovers (berseem, crimson, Persian and squarrosum) under water availability and rainfed conditions was reduced by 52.3 and 44.7% in berseem, 68.5 and 79.4% in crimson, 59.7 and 65.4% in Persian, and 33.5 and 45.9% in squarrosum under undefoliated and defoliated plants, respectively. These results showed variability among clovers species to defoliation and rainfed conditions. The variation existing among clover species was attributed to the effect of agronomic applications on biochemical routes of relocation of photosynthates from vegetative to reproductive organs of the plant.

On a study carried out by Martiniello and Ciola (1995), the mean of seed yield components of six berseem genotypes growing by alternating water availability (irrigated vs rainfed) and harvest (defoliated vs undefoliated), decrease the following traits: heads per stem by 16.3 and 30.6%; seed heads by 10.5 and 11.8%; 1000-seed weight by 9.4 and 8.3%, respectively; an opposite trend was observed for HI (56.1% in rainfed and 16% in defoliated condition) (**Table 5**).

In perennial grasses (cocksfoot, perennial ryegrass and tall fescue) and legumes (lucerne, sainfoin and sulla), the mean (over a 4-year evaluation) seed yield reduction under alternating water availability (irrigated vs rainfed) was 42.1 and 36.4% (undefoliated vs defoliated under irrigated) and 47.5 and 63.1% (defoliated vs undefoliated under rainfed) in grasses and legumes, respectively. The effect of water availability in comparison to rainfed reduced seed yield of grasses and legumes by 46.2 and 46.7%, respectively under undefoliated and 51.2 and 69.1% under defoliated conditions (**Table 6**). The seed yield component traits were also influenced by water (irrigation vs rainfed) and defoliation (defoliated vs undefoliated) state. The seed yield component trait mostly influenced under favourable water availability and undefoliated conditions in grasses and legumes was seeds per inflorescence, about 29% lower than in unfavour-

Table 6 Mean and their standard error (SME), of seed yield and its seed components of perennial species (3 grasses: cocksfoot, perennial ryegrass and tall fescue; and 3 legumes: lucerne, sainfoin and sulla) evaluated under different weather and defoliation condition of growing in Mediterranean environment (Elaborated form Martiniello and Ciola 1994; Martiniello 1998).

Species $^{£}$	Seed yield ^{a} (± SEM)		Stem density ^{b} (± SEM)		Seeds inflorescence ^{c} (± SEM)		1000-seed weight ^{d} (± SEM)		HI ^{e} (± SEM)	
	Irrigated	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**	**Irrigated**	**Rainfed**
No defoliation										
Cocksfoot	362 ± 35	181 ± 25	394 ± 114	309 ± 130	82 ± 6	40 ± 4	1.3 ± 0. 2	1.1 ± 0.2	16 ± 4	14 ± 5
P. Ryegrass*	285 ± 32	171 ± 24	1121 ± 201	1131 ± 246	62 ± 5	55 ± 5	1.4 ± 0.1	1. 2 ± 0.2	14 ± 3	13 ± 4
Tall fescue	300 ± 27	158 ± 19	515 ± 94	374 ± 98	42 ± 7	36 ± 4	2.1 ± 0.2	1.5 ± 0.2	17 ± 3	14 ± 4
Lucerne	332 ± 23	184 ± 21	334 ± 14	271 ± 25	73 ± 5	55 ± 6	2.2 ± 0.2	1.9 ± 0.2	16 ± 5	16 ± 5
Sainfoin	975 ± 65	475 ± 65	523 ± 14	415 ± 31	37 ± 1	17 ± 4	13 ± 2	12 ± 5	18 ± 6	16 ± 5
Sulla	1365 ± 115	910 ± 10	316 ± 10	234 ± 36	23 ± 2	20 ± 5	4 ± 2	4 ± 2	15 ± 5	15 ± 2
Defoliation										
Cocksfoot	286 ± 39	98 ± 28	311 ± 125	182 ± 143	65 ± 7	24 ± 4	1.0 ± 0.2	0.6 ± 0.2	13 ± 4	8 ± 5
P. Ryegrass*	225 ± 33	84 ± 24	885 ± 201	667 ± 244	49 ± 5	32 ± 5	1.1 ± 0.1	0.7 ± 0.2	11 ± 3	8 ± 4
Tall fescue	237 ± 27	86 ± 19	406 ± 14	221 ± 98	33 ± 7	21 ± 4	1.7 ± 0.2	0.7 ± 0.2	12 ± 3	8 ± 4
Lucerne	263 ± 23	68 ± 21	123 ± 14	101 ± 25	58 ± 5	20 ± 6	1.8 ± 0.2	0.9 ± 0 .2	12 ± 4	6 ± 5
Sainfoin	685 ± 85	174 ± 180	194 ± 7	154 ± 5	13 ± 1	6 ± 1	15 ± 1	5.2 ± 0.2	26 ± 5	6 ± 1
Sulla	925 ± 185	337 ± 100	117 ± 4	86 ± 2	13 ± 1	7 ± 2	6 ± 1	2.2 ± 0.21	13 ± 2	6 ± 3

[£] Mean over two years and two variety for species: cocksfoot, Dora and Cesarina; *perennial ryegrass, Pamir and Vejo; tall fescue, Sibilla and Tanit; lucerne, Equipe and Romagnola; sainfoin, Zeus and Vala; sulla, Bellante and San Omero, evaluated in experiments under Mediterranean environments.

[a] kg ha^{-1}

[b] stems m^{-2}

[c] n.

[d] g

[e] %

Table 7 Effect four harvests during transition phonological stage of plant development on seed yield and its yield components in berseem clover variety: Sacromonte (Elaborated from: Martiniello *et al.* 1996).

Growth stage of plant development	Seed yield ^{a} (±SEM)	Heads plant ^{b} (±SEM)	Seeds head ^{b} (±SEM)	1000-seed weight ^{c} (±SEM)	HI ^{e} (±SEM)	Dead plant ^{e} (±SEM)
Fourth internodes	1.7 ± 0.2	35.6 ± 3.9	19.1 ± 0.9	2.6 ± 0.1	37 ± 1	48 ± 2
Eight internodes	2.1 ± 0.2	41.6 ± 6.2	19.9 ± 1.1	2.5 ± 0.1	26 ± 2	30 ± 5
Beginning of flowering	1.8 ± 0.1	40.2 ± 5.2	18.9 ± 1.1	2.6 ± 0.1	32 ± 1	15 ± 2
Seed maturity	1.5 ± 0.1	37.2 ± 6.7	15.1 ± 1.1	2.7 ± 0.1	37 ± 1	15 ± 1

[a] g plant^{-1}

[b] n.

[c] g

[e] %

able moisture conditions; others traits the mean reduction was 10.6% for stem density and 18.8% in 1000-seed yield (**Table 6**). By contrast, defoliation under rainfed strongly stressed the development of plants reducing the values of weather-favourable growing conditions by 37.8, 48.2, 48.5 and 33.3% in grasses and by 21.6, 60.7, 87 and 64.7% for stem density, seeds per inflorescence, 1000-seed weight and HI in legumes, respectively (**Table 6**). The different relationships among seed yield and its components in grasses and legumes (annual and perennial) under alternating condition of water availability and defoliation during the transition phase were ascribed to the effect of agronomic practices on biological pathways of relocation of photosythates from the organs of a plant to seed.

In berseem, Martiniello *et al.* (1996) found that defoliation at different phenological stages of plant development strongly influenced seed yield (**Table 7**). Defoliation at two phenological stages of plant growth (elongation until eight internodes and beginning of flowering) gave a seed yield of 19.1 and 28.6% in the former and 5.6 and 16.7% in the latter, respectively higher than defoliation in plants in which four nodes had elongated and in plants that were never defoliated (**Table 7**). Analogous results were observed by Martiniello and D'Emlio (1997) when lucerne was defoliated in two phenological stages of plant development (early and flowering) compared with undefoliated plants. Defoliation at an early stage increased seed yield in comparison to defoliation applied at the flowering and never defoliated by 9.3 and 7.3%, respectively.

Seed yield in forage crops is a compromise between plant development and the number of fructiferous organs at flowering. Defoliation in all cultivated forage crops for the purpose of seed production may be considered an agronomic tool able to maximize the effect of available environmental resources, balancing vegetative regrowth of stems and seed yield. Wrong use of defoliation may create drastic consequences on plant regrowth and on seed production. Early and late crop defoliation, as reported by Martiniello *et al.* (1996) in berseem may cause plant regrowth without harmony between vegetative and reproductive organs promoting variation in the turgor of cells during seed filling with a consequent interruption of the relocation of stored products in the seed. Investigations on regrowth after defoliation of forage crops species underlined the importance of management to safeguard axillaries buds, which, if damaged, would reduce regrowth and the number of stems.

The biomass of forage crops for grazing, hay and silage consumption is metabolized by physiological processes that occur between vegetative (seedheads) to transition (beginning of floral harvest induction) phenological phases of plant growth (Rawnsley *et al.* 2002; Rochon *et al.* 2004; Turner *et al.* 2006a; Martiniello 2009; Voler *et al.* 2009). The metabolic processes in the stem and leaf during the transition phase are governed by night and day temperatures, water stress and photoperiod which gradually induce the apical meristem of a vegetative bud to induce flowers in the reproductive conversion phase (Siddique *et al.* 2001; Martin *et al.* 2004; Iannucci *et al.* 2007; Sanada *et al.* 2007; Farooq *et al.* 2009; Trethewey *et al.* 2009). The morphological and physiological processes that occur in forage crops after defoliation have been studied in a project coordinated by Hannaway (2000) on "Grass growth and regrowth for improved management" at the Oregon University, USA. Plants, after defoliation, develop and growth new stems and leaves from crown buds utilizing the carbohydrate reserve (sugars and starches) or from active photosynthesizing leaves remaining in the stubble. During the vegetative stage, tillers continue normal development or until defoliation of the apical meristem remains intact; injury of the apical meristem implies a reduction and recovery of new tillers and the reserve products in the plant's organs will be delayed. Knowledge regarding the morphological position and specific function of axillary meristems on the crown is important for successful agronomic management. The health and vigor of stem regrowth after defoliation will depend on the full activity of an axillary meristem. The gain of regrowth in forage crops depends on basic information of defoliation management regarding the phonological stage of tiller development before floral induction which influences the quality of the biomass and the height of stem harvest from the ground level in the way to avoid damage of axillary buds on crown.

Cool-season forage crops require low temperature for inducing vernalization and increasing photoperiod which take place in the growing point and leaf, respectively (Chastain and Young 1998; Del Pozo and Aronson 2000; Zhang *et al*. 2006). Low temperature induces physiological changes that prepare the plant for the transition phase and initiate floral development. However, in perennial ryegrass, colonial and creeping bentgrass, a short day photoperiod can substitute vernalization while in warm-season forage crops flower induction occurs in a short day photoperiod when the plant remains in winter vegetative stasis. During the cycle of plant growth, vernalization induces an auxin, cytokinin and gibberellin requirement in the growing point for sustained activity of axillary buds and plant development in the transition phase while in leaves day length photoperiod induces variation of the hormones which favour flowering stimulus and translocation of photosynthates to the seed (Corbesier and Coupland 2006; Hay 2006; Bruns 2007; Rameau 2009; Pin *et al*. 2010).

The physiological activities of regrowth stems, leaves and roots are influenced by the available labile C and N in soil uptake. Morgan *et al*. (2001), under a controlled environment, examined regrowth biomass partitioning and labile C and N metabolites in three plant types: grass western wheatgrass and lucerne (C_3 plants) and blue grama grass (C_4 plant). Plants were seeded by adopting a factorial design arrange-ment of two labile CO_2 (low CO_2, 355 µmol mol^{-1} and high CO_2, 700 µmol mol^{-1}] and two N nutrition regimes (low and high). Plants were watered twice a week with half-strength Hoagland's solution: low N solution containing 0 N; and the other half-strength Hoagland's with a high N solution containing 14 µmol ml^{-1} of N. The authors evaluated the par-titioning of biomass in the organs of plants by the allelometric growth coefficient (AGC), which represents the balance between shoot and root relative growth rate by providing an estimate of the effect of CO_2 and N regimes on the partitioning response of build-up labile C and N components in the tissues. When AGC = 1, there is equal relative root and shoot growth; when AGC < 1, relative root growth was higher than shoots; when AGC > 1 the trend was opposite, i.e., root growth was lower than shoots. The biomass samples used for determining AGC were clipped tillers and roots at 10 and 20 days after the 4th defoliation. Samples of previous defoliations were not considered because modification of labile reserves was active during this period. High N enhanced regrowth in all three species while high CO_2 enhanced regrowth only in blue grama grass. In lucerne, CO_2 and N treatments had no significant effect on AGC. In contrast, AGC was reduced in western wheatgrass grown under low N than under high N. In blue grama grass, low N reduced AGC but interacted with high CO_2. AGC was greatest for plants grown under high N and CO_2 and high N and low CO_2; intermediate at low N and CO_2; and least at low N and high CO_2. These results indicate greater partitioning in the root when N is limiting, particularly under higher CO_2. The significant correlations established between AGC and several measurements of plant N status suggest that the effect of CO_2 on plant biomass partitioning involves the status of N.

The morphological position of meristematic axillary buds on the base of the plant is typical for each forage crop and is influenced by agronomic management (seed sowing and density) and particularly by the height of stubble left by defoliation of the plant during the transition phase (Boe and Mitchell *et al*. 2007; Miser and Nelson 2007; Bortnem 2009). Thus, successful agronomic exploitation of a forage crop needs accuracy and precaution in management during the defoliation of a plant, i.e. height of stubble and the plant, before floral induction. Malpractice during defoliation may destroy the crown meristems by reducing bud production as a consequence of a reduction of tillers, adventitious roots, rhizomes, stolons and storage organs which serve as carbohydrate reserves to support the regrowth of new plant organs.

Harvest index

The relation between grain yield and plant phytomass is commonly defined as harvest index (HI). It measures the ability of a plant to transfer photosynthetic compounds to temporary sink organs prior to flowering and subsequently their reallocation, as structural assimilates, to seed during grain filling. The value of HI among forage crops bred for agronomic purposes is quite variable.

The variability among forage crop species (berseem (Martiniello and Iannucci 1998); lunceme (Bolaños-Aguilar *et al*. 2002); sulla, sainfoin and harding grass (Martiniello 2005), elephant grass (Zhao *et al*. 2008) and fenumgreek (Basu *et al*. 2009)) to relocate assimilates from temporary organs to permanent sinks is a consequence of genetic variability that exists in seed yield components among varieties.

Studies on genetic variability to relocate stored compounds from the stem and leaves to seed and its yield components have been made on different forage crops.

Bolaños *et al*. (2002), in experiments on lucerne, reported variation in seed yield induced by genetic and environmental factors. The traits evaluated in the study were biomass, HI and seed yield components in 12 cultivars over 4 locations in 3 years of experiments. Seed weight, number of pods per inflorescence, number of seeds per pod and seed weight were also evaluated. Large variations in seed yield were found among cultivars and environments. Seed yield was highly correlated with biomass (r = 0.94) and HI (r = 0.99) and negatively with lodging (r = -0.89). The seed weight per inflorescence showed high broad sense heritability (h^2 = 58) and high genetic correlation with seed yield (r = 0.91) and with HI (r = 96). Variation in seed weight per inflorescence was associated with the number of seeds per pod and the number of pods per inflorescence. Seed weight was genetically strongly associated with number of seed per fructiferous organ and seed yield. The strong association discovered among HI and seed yield with its yield components evidences a relocation of stored compounds of biomass to seed organs of the inflorescence.

Selection programs based on autochthonous germplasms of sulla, sainfoin and harding grass accessions, carried out by Martiniello (2005), released breeding genotypes more adapted to weather conditions of the Mediterranean climate and productive in biomass and seed yield than basic accessions. From 6 Mediterranean environments about 1500 accessions of three grass species were collected and used as row material for three cycles of phenotypic recurrent selection. Crosses among accessions were made under natural mating conditions. Seeds of each basic accession were evaluated and selected in two contrasting environments: vegetative characteristics of row-plant plots and reproductive traits of spaced-plant plots. The tillers in the row-plot, at the flowering stage, were defoliated and biomass characteristics were determined. The accessions with higher biomass production were selected and, at physiological maturity of seed, plants of the space-plant plot were harvested and threshed; the cleaned seed was weighed. Seeds of each accession were used to make a bulk cycle population (equal weight of seed for each accession) and for preparing row-spaced and space-plant plots for evaluation of vegetative and reproductive states, respectively. Three cycles of selection were

Table 8 Comparison between harvest index mean of ecotypes and variety of forage crop species evaluated under alternating water, seed sowing density (Elaborated from: Martiniello and Ciola 1995 annual legumes, and perennial legumes by Martiniello and Ciola 1994).

Forage crop species	**Genotype tested** [a]	**Harvest index** [b]			
		Irrigated (Duncan's Multiple-Range Test)		**Non-irrigated (Duncan's Multiple-Range Test)**	
		High seed density	**Low seed density**	**High seed density**	**Low seed density**
Annual legumes					
Berseen					
Ecotype	7	10.9 a	12.6 a	9.4 a	8.7 a
Variety	4	8.3 b	10.4 b	7.4 b	7.1 b
Field bean					
Ecotype	5	2.9 a	2.8 a	4.1 a	4.1 a
Variety	2	2.7 b	2.8 a	3.8 b	4.4 a
Vetch					
Ecotype	5	7.2 a	10.1 a	10.4 a	10..3 a
Variety	2	7.8 a	8.9 b	8.9 b	11.3 a
Perennial legumes					
Sainfoin					
Ecotype	2	20.2 a	17.0 a	8.9 a	8.6 a
Variety	2	15.8 b	16.0 b	7.6 b	7.2 b
Sulla					
Ecotype	2	20.5 a	24.9 a	31.1 a	30.1 a
Variety	3	9.2 b	11.0 b	12.1 b	10.2 b

[a] n.
[b] %

developed from this breeding program: 1050, 700 and 550 accessions were selected in the first, second and third cycle of selection, respectively and 70% selection pressure was adopted for all three cycles. The increase of dry matter yield made by selections (genetic gain) from the first to the second and third cycle (almost similar among species) was about 7, 6 and 3%, respectively. This breeding method could select accessions with higher biomass and yield potential than those used at the beginning of the experiment. The mean biomasss and seed yield trait of improved and basic accessions was 15.4 and 7.9% in sainfoin, 13.9 and 13.3% in harding grass and 15.5 and 6.7% in sulla, respectively. The agronomic evaluation of the selected breeding populations named Irpinia for sulla, Tetim for sainfoin and Partenope for harding grass, over four Mediterranean environments, resulted in better adapted and more productive, i.e., higher biomass and seed yield (34.5 and 63.4% in sainfoin, 28.2 and 32.5% in harding grass and 24.5 and 25.7% in sulla, respectively). The high seed yield of the released breeding populations of the three species may be a consequence of translocation of solutes stored in temporary organs to the seed. The agronomic field evaluation, heritability and stability of the three breeding populations (Tetim, Irpinia and Partenope) provides evidence that the breeding method used was able to release genotypes with more efficient physiological pathways in transferring phothosynthates in plant organs better than varieties tested in Mediterranean environments.

Zhao *et al.* (2008) evaluated non-structural carbohydrate (NSC, i.e., glucose, fructose, sucrose, starch and fructans) variation in cool-season grasses: four tall fescue, two *Festulolium* (*Lolium multiflorum* x *Festuca arundinacea*), one tall wheatgrass, two intermediate wheatgrass and four smooth bromegrasses. Grass species influenced the concentration of NSC, crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) in the range of 70 to 112, 81 to 138, 548 to 614 and 288 to 321 g kg^{-1} dry weight, respectively. The difference among the relative contribution of each carbohydrate component to total NSC was small among the five species. The average glucose, fructose, sucrose and starch accounts for 14, 10, 15, 38, and 23% of total NSC, respectively. The concentration of NSC in the sink organs represents the biochemical energy which can be used for forage consumption or for relocation in the sink organ for increase seed production.

Zhao *et al.* (2010) developed a laboratory-based method to determine NSC composition and concentration for determining the resources available for plant growth and biochemical energy using the cool-season forage crops of the 2008 study, which can be used for forage or seed production. The authors adopted a kit consisting of microplate enzymatic assay to evaluate the NSC components. The kit provides detailed information about reagents, proportions of chemical solution and the optimal condition for the reaction for each NSC component.

The genetic base of the relocation of stored compounds in biomass to seed sink was determined by qualitative trait loci (QTLs). A study was established for two years in a field experiment by Gondo *et al.* (2007) by analysis of multiple agronomic traits in the model legume *Lotus japonicus* (Regel) K. Larsen) performed with a population of recombinant inbred locus derived from Miyakojima MG-20 x Gifu B-129. Thirteen agronomic characteristics were evaluated: vegetative part of plant traits (plant height, stem thickness, leaf length, leaf width, plant growth, plant shape and stem colour), flowering traits (flowering time and degree), and pod and seed traits (pod length, pod width, number of seeds per pod and seed mass). A total of 40 QTLs were detected which explained 5 to 60% of total variation. The QTL that explained the most variation was for stem colour, which was detected in the region of chromosome 2. Some QTLs, especially those for pod and seed mass, had 5 locations that mapped to the corresponding genomic positions of equivalent QTLs in soybean, pea, chickpea and mung bean.

Unfortunately, the advantage of new methodologies that can derive from their application in breeding forage crops for seed production were not take into considerable account by the Italian and EU law [Ministero Politiche Agricole, Alimentare e Forestale (1971, 2008); European Commission (2003, 2010)], which determined that in forage crops, the assessment of seed yield trait, during the inspection of a newly released variety, is not a mandatory requirement in agronomic field evaluations; thus, public and private Institutions, during the selection process of a variety, did not include this trait as a main target in breeding programs (Rotili *et al.* 1996; Simmon 1996; Falcinelli and Martiniello 1998; Rossellini 2004). For these reasons, forage crop breeding efforts have been based on increasing the biomass yield potential of cultivated plants by improving biomass yield rather than seed yield (**Tables 1, 4, 8**) (Casler *et al.* 2000; Iannucci *et al.* 2002; Rochon *et al.* 2004; El Karamany 2006).

Comparing the yearly genetic gain values of forage and seed production (**Table 4**) and those of HI among cultivated forage crops and ecotypes with those of varieties mainly diffused in agriculture (**Table 8**), it is possible to assume that efforts in genetic selection made to promote an increase in seed production have been lower than those to increase dry matter yield.

The objectives of breeding programs give more emphasis to increasing plant parts with greater economic interest rather than to improve the efficiency of metabolic processes involved in phytomass and seed production. However, because seed yield is not emphasized in the aims of the breeding program, the HI values in forage crops, as those reported in **Tables 4** and **8**, showed low values among forage crops and a narrow range of variation when compared with those of other crops (Baenziger *et al.* 2006). Thus, the genetic gain for HI in varieties released in the last 10 years, in comparison to HI values of old varieties, was inconsistent. The reasons for low genetic gain for seed yield and its yield components were a low correlation with herbaceous traits that eliminated the genetic effect of selection made for improving biomass which does not imply an increase in seed yield improvement programs for variety release have to give similar priority to biomass and seed production. Thus, old varieties still present higher seed yield than newly released cultivars (**Table 4**). By contrast, breeding efforts for biomass production in newly released varieties in the last decade, in comparison to old landraces, was quite different among forage crops (**Table 1**).

The lack of genetic gain in seed production was due to the fact that seed yield assessment is not requested as minimum trait during the field agronomic evaluation of released forage crop varieties candidate to be included in the list of the European Catalogue Register of States that belong to the European Union (Ministero Politiche Agricole, Alimentari e Forestali 2008; European Commission 2010).

In other countries around the world, the registration of a newly released variety is governed by international rules established by non-governmental organizations (NGOs) such as the International Seed Testing Association (ISTA), the Union for the Protection of New Variety of Plant (UPOV) and Plant Breeders' Rights (PBR), which have published international guidelines for governing how agronomic and laboratory tests have to be conducted on DNA profiles and on plants for determining the distinctness, uniformity and stability (DUS) of traits in new released varieties (Loch and Ferguson 1999).

Number and size of seed in reproductive organs

The number of seed in fructiferous organs is related to pollination. In grasses pollination depends mainly on moderate wind during flowering (up to 40%), while in legumes on the availability of appropriate pollinators (Fairey and Lefkovitch 1993; Martiniello *et al.* 2003; Pinzauti and Martiniello 2006). The potential number of seeds present in a fructiferous organ depends on environmental resources and management practices used during the vegetative phase of development. The use of wild pollinators, reproduced in the laboratory, facilitates pollen exchange, reduces the period of exposure of mature pollen eggs to the risk of environmental factors and increases the number of seed per fructiferous organ (Strickler and Richards 1997; Meffe 1998; Satta *et al.* 2000; Martiniello *et al.* 2003).

A field experiment was carried out by Martiniello *et al.* (2003) to assess the effect of wild pollinators in seed production in two forage crop varieties well diffused in the cultivation of Mediterranean environments. The variety used in the experiments belongs to berseem and lucerne species and the pollinator was the leafcutter bee (*Megacile rotundata* F.) and the mason bee (*Osmia cornuta* Latr.). The pollinators were reproduced in an insect management laboratory according to the procedures of Pinzauti (2000) and Felicioli (2000). When the crops flowered, a woody protective structure of cocoons with pupae ready to release emerged adult insects and a woody nest-trap for rearing were laid in the centre of the field experiment. The adult female activity stored the pollen grains gathered during the visit of flowers and lodged the eggs, separating them one by other from round disks, in the tunnels of woody artificial nest. The top and bottom of rearing cylindrical core built by round disks of leaves, pollen and eggs was hermetically sealed and contains an amount of alimentary reserve (protein and sugars) enough for sustaining the metamorphosis of pupae during winter under controlled environment. At end of pollination, the nest trapping from field experiment were transferred in the entomological laboratory where the nests were opened, cocoons selected and entomologically manipulated for preparing their release in further pollination.

The effect of pollinator on seed production was determined using plants harvested at physiological maturity of seed in the fructiferous organ every 10 m on transects built on six diagonal drawn from centre and edges of two side field experiment with insect releasing-rearing woody structure. The mean of seed yield achieved by using the leafcutter bee in crop management of lucerne and berseem clover was 4.4 and 4.1 g m^{-2} instead of 2.9 and 3.2 g m^{-2} during production obtained by natural pollination, respectively (Martiniello *et al.* 2003). The wild pollinators increased more than the control without any pollinator, seed yield by 35.5 and 13.6%; heads per stem by 31.3 and 18.5%; seed per fructiferous organ by 24.1 and 11.6% in lucerne and berseem, respectively. The gain in seed yield due to insect activity took place in the third to tenth transects. The low seed yield in the transects near the point of release and after the 10th transect was due to the damage caused to leaves and flowers of plants caused by foraging insects and a reduction of visiting activity from the 10th transect. The wild insects used in the experiment for both forage crops induced variation in seed set for fructiferous organs attributable to the activity of pollinators which facilitated pollen exchange among plants.

Analogous results were reported in Canada when insect pollinators were employed during flowering, indicating the possibility of achieving a substantial increase (> 35%) in seed yield (Fairy and Lefkovitch 1993; Martiniello *et al.* 2003; Hanlin *et al.* 2007).

The agronomic advantages favoured by the use of insect pollinators are not diffused in the management of seed industry production in forage crops (Kroskor *et al.* 2006). The reduced use in management practices in seed industry production is ascribed to the scant requirement of wild insects and service facilities which hinder their utilizations as routine in agronomic management (Karise *et al.* 2006; Klein *et al.* 2007; Dewenter and Westphal 2008).

The use of insects for pollination in confined environments and for breeding pollination crops has been not well exploited. Their use as cross breeding pollinators has been found to be extremely efficient in breeding programs for variety release (Piano *et al.* 1998; Pecetti and Romani 2003; Pinzauti and Martiniello 2006). Wild insects (leafcutter bee and mason bee) and domesticated honeybee (*Apis mellifera* L.) favouring pollination reduce the pollination period and consequently lower the risk of crops to atmospheric agents increasing the fertility of fructiferous organs (pods or heads) of berseem, lucerne and sulla (Martiniello *et al.* 2003; Pecetti and Romani 2003; Pinzauti and Martiniello 2006). Furthermore, the use of a high number of insects increases the possibility that visiting flowers be pollinated before the natural decline of the stigma whose receptivity is accelerated reducing environmental risk of adverse weather conditions (high temperature or hot wind) on receptivity of stigma to pollination during flowering (Fairey and Lefkovitch 1993; Satta *et al.* 2000; Martiniello *et al.* 2003; Hanlin *et al.* 2007). The use of pollinator insect agriculture for seed industry production and in plant breeding will enhance seed production and breeding activity in forage crops.

Seed development in the reproductive growth phase

The pollination percentage of ovules (60-80% of florets) in both grasses and forage legume crops represent a critical aspect of seed production. Most of the pollinated egg mother cells may desiccate with a consequent abortion of developing seeds. The exploitation of forage crops presents two distinct aspects that are closely linked: 1) biomass production for grazing, hay and silage consumption and 2) seed for genotype reproduction. Scientific agronomic studies have focused effort on biomass production while those on seed production, although the most important for reproduction of genotypes, particularly in EU member states, have not received due attention (Simon 1996; Martiniello 1998a; Armstead *et al*. 2008).

The agronomic activity of seed industry production depends on appropriate management techniques for meteorological events of Mediterranean environments which influence the physiological mechanisms of relocation of photosynthates during the seed-filling period (Martiniello and D'Emilio 1997; Iannucci *et al*. 2000b; Bolaños-Aguilar *et al*. 2002; Sengul 2006). WUE may be used as a criterion for evaluating the efficiency of biological mechanisms which underlie the relocation of photosynthates in the storage organs of a plant (Stanhill 1987). Martiniello (1998a, 1999) found that, in experiments carried out in Mediterranean environments, the efficiency of physiological mechanisms for seed production, in annual and perennial forage crop species, are better expressed under favourable water availability (**Table 3**).

Similar conclusions have been drawn in the literature regarding the effect of water deficit and defoliation on the physiological pathway of relocation of photosynthates in the seed of forage crops. Studies carried out in environments with Mediterranean and Mediterranean-like climates include lucerne (Martiniello and D'Emilio 1997; Iannucci *et al*. 2002), berseem, perennial ryegrass, Persian clover, red clover, sainfoin and sulla (Martiniello 1998a, 1999), narrow-leaf lupine, and yellow lupine (Palla *et al*. 2004), groundnut (Clevel *et al*. 2005), pea, field bean, lentil, chickling vetch, ochrus chickling, grass pea, narbon bean, common and purple vetch (Siddique *et al*. 2001) and corn (Lauer *et al*. 2004).

The lower differences in WUE observed among forage crop species were ascribed to physiological mechanisms which enabled the plants to maintain high osmotic adjustment of cytoplasm in embryo and endosperm cells. According to Iannucci *et al*. (2002) and Zhang *et al*. (2008), the physiological discrepancy observed under growing conditions may be due to soil water availability during plant development. Water applied by irrigation during crop growth was utilized by the plant to reduce the effect of environment on the physiological process of photosynthate relocation to seed. The differences observed in the WUE of seed yield of irrigated and rainfed conditions among of forage crops species may be due to the osmotic adjustment of cells to tolerate seed desiccation (**Table 3**).

Influence of environmental factors on seed production and seed yield components

Phytomass production for livestock consumption depends on the availability of seed in the international market. In the 20th Century, genetic gain in seed yield of forage crops due to plant breeding is estimated to be 4% decade^{-1} lower than other grain crops (13.5% decade^{-1}) (Claser *et al*. 2000; Barret *et al*. 2005; Baeziger *et al*. 2006). In grain cereal crops, the major contribution to variation has been HI whose increase was due to seed gain over plant phytomass. In contrast, new varieties of forage crops have poor seed production indicating that breeding selection for this trait received weak scientific attention compared to the attention given to phytomass production (**Tables 2, 3**) (Simon 1996; McCromack 2004; Humphreys 2005; Baenziger *et al*. 2006; van der Heijden and Roulund 2010).

The increase of genetic gain of seed yield requires more effort in a breeding program that considers the improvement of forage and seed yield traits melted together in an objective applying selection procedure in multiple contrasting locations selecting for herbage and seed yield, seed weight and the number of seed per fructiferous organ as applied in lucerne (Bolaños *et al*. 2001), in sulla, sainfoin and harding grass (Martiniello 2005) and fenugreek (Basu *et al*. 2009). Thus, nowadays, an increase in seed production in forage crops can be achieved using appropriate management techniques (biotechnology and molecular genetics) able to improve and optimize the components of seed yield which favour exploitation of the genetic potential of varieties and avoid environmental stress (Murphy 2007; Boelt and Studer 2010; Kölliker *et al*. 2010).

Experiments carried out by Martiniello (1998, 1999) and El Karamany (2006) found that variation of water availability during the vegetative cycle of crops influenced seed yield and seed yield components (**Table 4**). Irrigation experiments (Martiniello and Ciola 1995; Martiniello 1998, 1999; Steiner *et al*. 2006) evidenced that a reduction in seed yield under rainfed condition may range from 40 to 50% in perennial grasses (cocksfoot, tall fescue and perennial ryegrass), 45 to 83% in perennial legumes (lucerne, sainfoin and sulla) and 10-60% in annual clovers (berseem, crimson, Persian and squarrosum).

Furthermore, seeds/stem and seed weight were the yield components most affected by environmental factors. Martiniello (1998b) reported that available water during the seed-filling period showed a positive significant correlation between seed yield and stem density and seeds/stem. In agreement with Annicchiarico *et al*. (1999) and Alves de Araújo and Coulman (2004), the correlation (r) values between seed yield and seed weight under irrigated conditions always exceeded those of rainfed (respectively: 0.87 and 0.60 in cocksfoot; 0.95 and 62 in perennial ryegrass, 0.71 and 0.62 in sainfoin; and 0.85 and 0.82 in lucerne). The higher r values for irrigation than rainfed denote different relationships among seed yield components in determining seed production. Thus, it is possible to postulate that in Mediterranean environments, water resources favour metabolic routs of relocation products to seeds and reduced the damage of physiological processes for transferring photosynthates to seed fructiferous organs (Martiniello *et al*. 1996; Iannucci *et al*. 2000b; Bolaños-Aguilar *et al*. 2002).

In some clovers (berseem, crimson and Persian), as reported by Martiniello (1999) under defoliated and non-defoliated growth conditions, the correlation between phytomass and seed yield under water availability, was lower under undefoliated (r=0.32) than defoliated (r= 0.64) conditions. A similar correlation was found by Annicchiarico *et al*. (1999) in white clover and Alves de Araújo and Coulman (2004) in meadow bromegrass.

The different r values are attributed to metabolic activity of regrowing plants to remobilize phosynthates into reproductive organs rather than to vegetative stems and leaves. Defoliation experiments at different phenological stages of plant development (Martiniello and D'Emilio 1997; Todorovic *et al*. 1999; Iannucci *et al*. 2002) evidenced that the metabolic routes of stored compounds in organs (root, stem and leaf) was related to the phenological stage of plant development.

From an agronomic point of view, plant regrowth after defoliation made at an early flowering stage may have a higher probability of reducing soil evaporation and favour the relocation of stored compounds (protein and total available carbohydrates) to seeds. In berseem, the content of biochemical compounds stored in the organs of a regrowing plant in early flowering is able to support the development of the seed-filling period more efficiently than defoliation applied at a late stage of plant development (Iannucci *et al*. 1996; Martiniello *et al*. 1996).

Further studies on the effect of defoliation on regrowth stem of grasses (cocksfoot, perennial ryegrass and tall fescue) and legumes (lucerne, sainfoin and sulla), on seed yield, seed weight and seed per fructiferous organs under rainfed and irrigated growth conditions were conducted by Matiniello (1988) and Iannucci *et al*. (2002) (**Table 6**). The physiological and genetic basis involved in the relocation of photosynthates from vegetative to reproductive organs of plants has been described by Trethewey and Rolston (2009) and Armstead *et al.* (2008), respectively. Trethewey and Rolston (2009) studied the effect of defoliation on plant regrowth on relocation of water-soluble carbohydrates (WSCs) in perennial ryegrass. The authors applied defoliation and determine the contribution of total amount of carbohydrates present in the vegetative part of the plant to seed yield. The pattern of accumulation of WSCs and their remobilization was investigated in field-grown plants periodically defoliated from the beginning of early heading through to harvest. The amounts of WSC present in different parts of the plant (leaf blades, leaf sheaths and internodes) were measured in two ways: indirectly by changes in tissue dry weight and directly by determining the low molecular weight (LMW) and high molecular weight (HMW) carbohydrates in vegetative and reproductive tissue samples by quantifying the concentration in a colorimetric anthrone assay. High concentrations of HMW of WSC and dry matter weight were found in the internodes at post head emergence and these concentrations continued to increase during seed filling to harvest. In contrast, in the leaf blades and leaf sheaths the total dry matter decreased only slightly while the LMW of WSC steadily declined. In fructiferous organs, WSC increased significantly during seed filling and subsequently declined while dry matter continued to increase until harvest. The ratio of WSC LHW and HMW changed during tiller development. The variation depended on tissue type and growth stage indicating that the seed fructiferous organ is itself involved in driving the relocation of WSC mobilization from vegetative organs to seed.

The relocation of stored compounds in the organs of a plant to seed in two perennial ryegrasses is under genetic control (Armstead *et al.* 2008). The authors identified genetic components which control the relocation of stored reserves from vegetative parts to seed by QTLs.

The identification of QTLs associated with seed set in fructiferous organs as a major component of seed yield in perennial ryegrass indicates that mutational load associated with genes of the genomic regions suggest that there is selectable variation at this locus that could significantly increase seed-setting ability in industrial varieties. Thus, development of molecular markers for seed set genes will be useful for identifying favourable allelic variants with the aim of incorporating them into different breeding populations. QTLs for seed in the fructiferous organ were identified on chromosome LG7 in both ryegrasses and on LG4 only in one genotype. Comparative genomics suggests that LG7 and LG4 regions are syntenous to a region of rice LG6 which contains both fertility and heading candidate genes and LG3, with contains a candidate fertility gene. The identification of QTLs associated with seed in fructiferous organ as a major component of seed yield in perennial ryegrass indicates that mutational load associated with seed genomic regions can be mitigated through marker-associated selection.

The path coefficient analysis used by Iannucci and Martiniello (1996) in four annual forage clovers (berseem, crimson, Persian and squarrosum), Annicchiarico *et al.* (1999) in white clover, Sengul (2006) in lucerne and Zhang *et al.* 2010) in elephant grass provides information on the relationship among seed yield and seed yield component traits in 4 annual forage crop species. The analysis was applied to four clover species (berseem, crimson, Persian and sqarrosum), grown under rainfed and irrigated growth conditions. The results of path analysis showed that number of seeds/fructiferous organ, fructiferous organs/surface unit and seed weight were the components mostly influenced by irrigation treatment. Furthermore, because environments with a Mediterranean climate, forage crops with early flowering are potentially endowed to reach physiological maturity of seed when the weather characteristics present mild water and heat stress conditions which may reduce physiological process of grain filling and, as a consequence, reduce the risk of negatively interfering with seed yield. Thus, the results of analyses evidenced, in agreement with Oliva *et al.* (1994), Martiniello and D'Emilio (1997) and Bolaños-Aguilar *et al.* (2002), that metabolic processes in early flowering better exploit the available environmental resources and relocate the stored compounds to sink sites of the seed yield components. Thus, defoliation plays an important role in preparing shoot regrowth to develop a plant in harmony with environmental conditions of the available weather resources for seed yield in forage crops.

The plant's phenological stage at the time of defoliation is influenced by the efficient adaptability of a regrowing stem to environmental resources, water status in the organs of a plant for maintaining cellular turgor in an embryo and endosperm cells during the seed-filling period (Noquet *et al.* 2001; Avice *et al.* 2003). Thus, stems of a regrowing plant after defoliation have to develop as soon as possible to the optimum stage of development to reduce the loss of soil water by evaporation, rebuild stored compounds before flowering in temporary organs and to maintain optimal water potential in embryo and endosperm cells. Particularly, in environments characterized by a Mediterranean climate, appropriate management techniques enable forage crops to exploit genetic potential and increase the production of phytomass and seed (Cane and Schiffhauer 2003; Martiniello *et al.* 2003; Hanlin *et al.* 2007).

Plant seeding rates influence plant growth rather than seed production. In environments with poor weather resources, sowing rate plays a secondary role in determining stems at harvesting time. Results obtained on two seeding densities in annual and perennial forage crops by Martiniello and Ciola (1993 and 1994) and Borreani *et al.* (2007) evidenced that differences between seeding rates had a marginal effect in both phytomass and seed production. The effect of seeding rates on a regrowing plant after defoliation disappeared. The lack of plant density on stem regrowth was ascribed to competition of the plant for natural resources (Zannone *et al.* 1995; Rotili *et al.* 1996).

CONCLUSIONS AND REMARKS

The genetic gain for seed yield traits in varieties released in the last 10 years has been inconsistent. The diffusion of forage crop variety in agriculture is linked to seed availability on the market.

The failure of breeding selection for seed yield improvement may have to be recovered by developing appropriate agronomic techniques able to reduce the effect of environmental impacts on physiological processes involved in seed production.

Environmental factors, like water availability and high temperature, have a direct impact on plants, causing damage of metabolic processes during vegetative and reproductive plant development with a consequent drastic reduction of biomass and seed production.

Efforts to understand the morphological, physiological and biochemical events during vegetative and reproductive phases are useful to reduce the impact of the environment on biomass and seed production. Reviewing the factors which influence the development of plants, from seeding to seed maturation, is useful for planning agronomic management techniques able to exploit the genetic potential of forage crops and environmental resources.

Knowledge of physiological processes and information about the metabolic pathways in the relocation and partitioning of photosynthates in the organs of plants makes it possible to plan appropriate management techniques to better exploit the genetic potential of phytomass and seed production. Furthermore, identifying the plant and seed physiological mechanisms which control water status in plant organs also makes it possible to add value to environmental resources and to stabilize phytomass and seed production of cultivated forage crops.

The utilization of new knowledge on plant physiology and molecular biology combined with breeding methodologies may provide forage crops genotypes endowed with biochemical peculiarities able to exploit the available resources of Mediterranean environments.

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Appendix List of common (alphabetical order) and scientific name and botanical family of genotypes used in the review.

Common name	**Scientific name**	**Botanical family**
African Isenberg grass	*Sporobolus stapfianus* Gand.	Poaceae
Aikiaki	*Sporolobolus virginicus* (L.) Kunth	Poaceae
Alsike clover	*Trifolium hybridum* L.	Fabaceae
Altai wildrye	*Leymus angustus* (Trin.)	Poaceae
Annual bluegrass	*Poa annua* L.	Poaceae
Bahiagrass	*Paspalum notatum* Flüggé	Poaceae
Beach vitex	*Vitex rotundifolia* L.f.	Verbanaceae
Bermudagrass	*Cynodon dactylon* L.	Poaceae
Berseem	*Trifolium alexandrinum* L.	Fabaceae
Birdsfoot trefoil	*Lotus corniculatus* L.	Fabaceae
Blue grama	*Bouteloua gracilis* (Willd. ex Kunth) Lag. ex Griffiths)	Poaceae
Bromegrass	*Bromus inermis* Leyss.	Poaceae
Buckwheat	*Fagopyrum esculentum* Moench	Polygonaceae
Bulbous barley	*Hordeum bulbosum* L.	Poaceae
Bulbosus bluegrass	*Poa bulbosus* L.	Poaceae
California burclover	*Medicago polymorpha* L.	Fabaceae
Canola	*Brassica napus* L.	Cruciferae
Carrot	*Daucus carota* L.	Apiaceae
Chickpea	*Cicer arietinum* L.	Fabaceae
Citrus	*Citrus reticulata* (Blanco)	Rutaceae
Chickling vetch	*Lathirus cicera* L.	Fabaceae
Cocksfoot	*Dactylis glomerata* L.	Poaceae
Colonial Bentgrass	*Agrostis tenuis* Sibth.	Poaceae
Common vetch	*Vicia sativa* L.	Fabaceae
Corn	*Zea mays* L.	Poaceae
Cotton	*Gossypium hirsutum* L.	Malvaceae
Creeping Bentgrass	*Agrostis palustris* Huds.	Poaceae
Creeping meadow foxtail	*Alopecurus arundinaceous* Prior.	Poaceae
Crimson clover	*Trifolium incarnatum* L.	Fabaceae
Darnel	*Lolium temulentum* L.	Poaceae
Desert wheatgrass	*Agropyron desertorum* (Fischer ex Link) J.A. Schultes	Poaceae
Dwarf Brazilian banana	*Musa acuminata* Colla.	Musaceae
Elephant grass	*Pennisetum purpureum* Schmach.	Poaceae
Fenugreek	*Trigonella foenum-gracearum* L.	Fabaceae
Field bean	*Vicia faba* L.	Fabaceae
Grass pea	*Lathirus sativus* L.	Fabaceae
Groundnut	*Arachis hypogea* L.	Fabaceae
Hairy vetch	*Vicia villosa* Roth.	Fabaceae
Harding grass	*Phalaris aquatica* L.	Poaceae
Indian fig	*Opuntia ficus-indica* Mill.	Cactaceae
Italian ryegrass	*Lolium multiflorum* Lam.	Poaceae
Intermediate wheatgrass	*Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey	Poaceae
Kawelu	*Eragrostis variabilis* (Gaudich) Steud.	Poaceae
Kentucky bluegrass	*Poa pratensis* L.	Poaceae
Klein grass	*Panicum coloratum* L.	Poaceae
Lampuaye	*Dodonaea viscosa* (L.) Jacq.	Poaceae
Lentils	*Lens culinaris* Medicus	Fabaceae
Little bluestem	*Andropogon scoparius* (Michx.)	Poaceae
Lucerne	*Medicago sativa* L.	Fabaceae
Meadow bromegrass	*Bromus riparius* Rehmann	Poaceae
Mexican liliac Glicicidia	*Gliricidia sepium* (Jacq.) Walp	Fabaceae
Mouse-ear cress	*Arabidopsis thaliana* (L.) Heynh.	Brassicaceae
Mung bean	*Vigna radiata* (L.) R.Wilczek	Fabaceae
Narbon bean	*Vica narbonensis* L.	Fabaceae
Narrow-leaf lupine	*Lupinus angustifolius* L.	Fabaceae
Oats	*Avena sativa* L.	Poaceae
Ochrus chickling	*Lathyrus ochrus* (L.) D.C.	Fabaceae
Panax	*Polyscias guilfoylei* (Bull ex Cogn. & E. March.) Bailey	Araliaceae
Paspalum	*Paspalum hieronymi* Hack.	Poacea
Pea	*Pisum sativum* L.	Fabaceae
Perennial ryegrass	*Lolium perenne* L.	Poaceae
Persian clover	*Trifolium resupinatum* L.	Fabaceae
Pili grass	*Heteropogon contortus* (L.) P. Beauv. Ex Roem. & Shult.	Poaceae
Pintoi peanut	*Arachis pintoi* Krap. & Greg. Nom. Nud. (Cook)	Fabaceae
Rhizoma peanut	*Arachis glabrata* Benth.	Fabaceae
Prairie grass	*Bromus willdenowii* Kunth.	Poaceae
Purple vetch	*Vicia benghalensis* L.	Fabacieae
Red clover	*Trifolium pratense* L.	Fabaceae
Red orache	*Atriplex hortensis* L.	Chenopodiaceae
Rice	*Oryza sativa* L.	Poaceae
Russian wildrye	*Elymus junceus* (Fisch.) Nevski	Poaceae
Sainfoin	*Onobrychis viciaefolia* Scop.	Fabaceae

Appendix (Cont.)

Common name	**Scientific name**	**Botanical family**
Sideoats Grama	*Bouteloua curtipendula* (Michx.) Torr.	Poaceae
Signal grass	*Brachicaria decumbens* Stapf	Poaceae
Sleepy morning	*Waltheria indica* L.	Malvaceae
Smoot bromegrass	*Bromus inermis* Leyess.	Poaceae
Smut grass	*Sporobolus indicus* (L.) R.Br.	Poaceae
Soft wheat	*Triticum aestivum* L.	Poaceae
Soft winter wheat	*Triticum aestivum* L. em. Thell.	Poaceae
Spreading club moss	*Selaginella lepidophylla* (Hooker & Greville) Spring	Selaginellaceae
Spreading orache	*Atriplex patula* L.	Chenopodiaceae
Squarrosum clover	*Trifolium squarrosum* L.	Fabaceae
Subterranean clover	*Trifolium subterraneum* L.	Fabaceae
Sulla	*Hedysarum coronarium* L.	Fabaceae
Sunn hemp	*Crotalaria juncea* L.	Fabaceae
Sunshine	*Chrysopogon zizanioides* (L.) Robert	Fabaceae
Swichgrass	*Panicum virgatum* L.	Poaceae
Tall fescue	*Festuca arundinacea* Schreb.	Poaceae
Tall wheatgrass	*Thinopyrum ponticum* (Podp.) Z.-W. Liu & R.-C. Wang	Poaceae
Thickspike wheatgrass	*Elymus lanceolatus* (Scribn. & J.G. Sm.) Gould	Poaceae
Timothy-grass	*Phleum pratense* L.	Poaceae
Tobacco	*Nicotiana tabacum* L.	Solanaceae
Tomato	*Lycopersicon esculentum* Mill.	Solanaceae
Tufted vetch	*Vicia cracca* L.	Fabaceae
Tumbling saltweed	*Atriplex rosea* L.	Chenopodiaceae
Unquillo grass	*Poa ligularis* Nees ex Steudel	Poaceae
Western wheatgrass	*Pascopyrum smithii* (Rydb.) A. Löve	Poaceae
White clover	*Trifolium repense* L.	Fabaceae
Wimmera ryegrass	*Lolium rigidum* Gaudin	Poaceae
Yellow lupin	*Lupinus luteus* L.	Fabaceae
Yellow sweet clover	*Melilotus officinalis* (L.) Pall.	Fabaceae