

## PLANT BREEDING AS TOOL TO CHALLENGE CLIMATIC CHANGES IN FORAGE PRODUCTION. A REVIEW

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### LA MEJORA GENÉTICA VEGETAL COMO HERRAMIENTA PARA AFRONTAR LOS CAMBIOS CLIMÁTICOS EN LA PRODUCCIÓN DE FORRAJE. UNA REVISIÓN

#### Historial del artículo:

Recibido: 13/06/2016  
Revisado: 09/09/2016  
Aceptado: 10/02/2017  
Disponible online: 08/11/2017

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ISSN: 2340-1672

Disponible en: <http://polired.upm.es/index.php/pastos>

#### Keywords:

Genetic improvement, C3 and C4 plants, forage plants, germplasm material, plant domestication, maize hybrids.

#### ABSTRACT

Literature was reviewed concerning climatic changes, especially increase in carbon dioxide concentration [CO<sub>2</sub>] and temperature during the last centuries, and connecting these changes with biochemical pathways in plants, mainly photosynthesis activity and respiration. Plant breeding has been successful in moving the genome of plants for selecting different characteristics, such as high oil and protein of the maize kernel, increase of yield in several crops along different eras, resistance to diseases and pests and adaptation to environmental conditions. Therefore plant breeding is proposed as a tool to challenge the climatic change through development of new varieties with complex traits adapted to future environments, such as higher temperatures and lower rainfall. Utilization of natural or induced polyploidy has demonstrated to be a good aid to increase vigor, yield, resistance to diseases, and adaptation of forage crops to stress. Development of synthetic varieties for forage plants seems to be a good option for achieving breeding goals in cross-pollinated crops. Hybridization between different species and within species is sometimes used for increasing the vigor, the adaptation to environmental stress and the yield of cultivars. Hybrids between two species (e.g., from genus *Lolium*) produce usually a high rate of aneuploid plants in the F<sub>1</sub> generation. Recurrent selection for raising the rate of balanced euploid plants in the F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>... generations of the allopolyploid hybrids is needed for getting stable varieties adapted to the target stress. Commercial hybrid seed is preferred to synthetics provided that the additional yield and features compensate the extra cost of seed production. Results of forage maize hybrids related to the worldwide cultivated "B73 × Mo17" hybrid, which were selected for earliness, are presented. Exploration of new C4 tropical species, such as Teff, likely will help to produce forage in poor watering conditions. The use of <sup>13</sup>C isotopic discrimination seems to be a good indicator to breed for water use efficiency (WUE) in C3 plants. Biotechnological tools, such as molecular markers, will facilitate to achieve breeding objectives sooner. Breeding suggestions for development of new varieties in C3 and C4 species adapted to future environments should consider the possible biotic and abiotic stresses generated by the climatic change. Automated phenotyping platforms are used for simulating stress environments. Domestication of wild species may be an alternative for developing a new crop to meet the requirement of environmental change. Crop domestication is a difficult task; however the use of rational strategies, including breeding for reducing the effect of limiting factors and strengthening favorable traits will help to achieve the desired objectives. Use of autochthonous germplasm as base material is crucial for providing genetic variability and genes adapted to environmental conditions.

#### Palabras clave:

Mejora genética de plantas, plantas C3 y C4, plantas forrajeras, bancos de germoplasma, domesticación de plantas, híbridos de maíz.

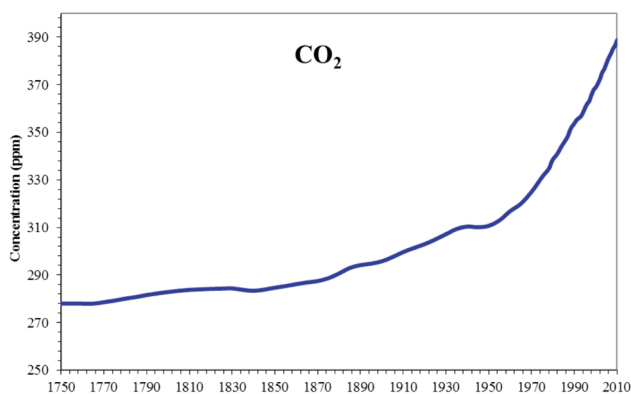
#### RESUMEN

Se revisó la literatura en lo referente al cambio climático, especialmente al aumento de la concentración de dióxido de carbono [CO<sub>2</sub>] y la temperatura durante los últimos siglos, así como su relación con las rutas bioquímicas en plantas, principalmente la actividad fotosintética y la respiración. La mejora genética de plantas ha tenido éxito en cambiar el genoma de las plantas seleccionando diversas características, tales como alto contenido de aceite y proteína en el grano de maíz, aumento del rendimiento en varios cultivos durante diferentes eras, resistencia a plagas y enfermedades y adaptación a las condiciones ambientales. Por lo tanto se propone a la mejora genética de plantas como una herramienta útil para afrontar el cambio climático mediante el desarrollo de nuevas variedades con caracteres complejos adaptados a los ambientes futuros. La utilización de la poliploidía natural o inducida ha demostrado ser una buena ayuda para aumentar el vigor, rendimiento, resistencia a enfermedades y adaptación de cultivos forrajeros a estrés. El desarrollo de variedades sintéticas en plantas pratenses parece ser una buena opción para alcanzar los objetivos de mejora en cultivos de polinización cruzada. La hibridación entre especies y dentro de especies gramíneas y leguminosas forrajeras se utiliza para aumentar el vigor, la adaptación al estrés y el rendimiento de las variedades. Híbridos entre dos especies (e.g. del género *Lolium*) producen generalmente una alta tasa de plantas aneuploides en la generación F<sub>1</sub>. La selección recurrente para elevar la tasa de plantas euploides (*i.e.* con equilibrio cromosómico) en las generaciones F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>... del híbrido alopoloide es necesaria para obtener variedades estables adaptadas al estrés. La semilla híbrida comercial se prefiere a las variedades sintéticas siempre que el incremento del rendimiento y otras características compensen el coste adicional de producción de semilla. Se presentan los resultados de híbridos de maíz forrajero relacionados con el híbrido ampliamente cultivado B73 x Mo17 y seleccionados para precocidad. La exploración de nuevas especies tropicales C4, tal como Teff, ayudará probablemente a producir forraje en condiciones de sequía y calor. El uso de la discriminación isotópica <sup>13</sup>C parece ser un buen indicador para mejorar la eficiencia en el uso del agua (EUA) en plantas C3. Las herramientas biotecnológicas, tales como marcadores moleculares, facilitarán alcanzar más pronto los objetivos de mejora genética. Se sugiere que la mejora genética durante el proceso de desarrollo de nuevas variedades en plantas C3 y C4 adaptadas a los ambientes futuros deberá tener en cuenta los estreses bióticos y abióticos generados por el cambio climático. La domesticación de especies silvestres puede ser una alternativa para obtener un nuevo cultivo que cumpla los requisitos del cambio ambiental. Plataformas automatizadas de fenotipado se utilizan para simular ambientes de estrés. La domesticación para desarrollar un cultivo es una tarea difícil; sin embargo el uso de estrategias racionales, incluyendo la mejora genética para conseguir eliminar factores limitantes y fortalecer los rasgos favorables ayudará a lograr los objetivos deseados. El uso de germoplasma autóctono como material base es esencial para proporcionar variabilidad genética y genes adaptados a las condiciones ambientales.

# 1. CLIMATE

## Concentration of CO<sub>2</sub> [CO<sub>2</sub>]

The change of atmospheric conditions is a tangible reality nowadays, characterized by an increase in the [CO<sub>2</sub>] as result of human activity, mainly caused by combustion of fossil fuel and cement emissions, as well as a negative balance of carbon storage in the terrestrial biosphere due to deforestation and breaking up of natural ecosystems. The same occurs in the oceans that have a negative CO<sub>2</sub> dynamic flow (Canadell *et al.*, 2007). The cement production has increased in the last 100 years due to the massive use in the building sectors of developed and developing countries. The cement manufacture contributes about 5 % to the increase of CO<sub>2</sub> in the atmosphere (Worrell *et al.*, 2001). About 65% of this contribution comes from the process of decarbonization of the limestone heated at high temperatures 1400-1500 °C releasing CO<sub>2</sub> and forming CaO; other 25% comes from the energy consumed for heating the furnaces up to those temperatures; and another 10% is due to the processes of extraction and transport of limestone.



**FIGURE 1.** Evolution of carbon dioxide concentration [CO<sub>2</sub>] in the atmosphere (Source: Environment European Agency).

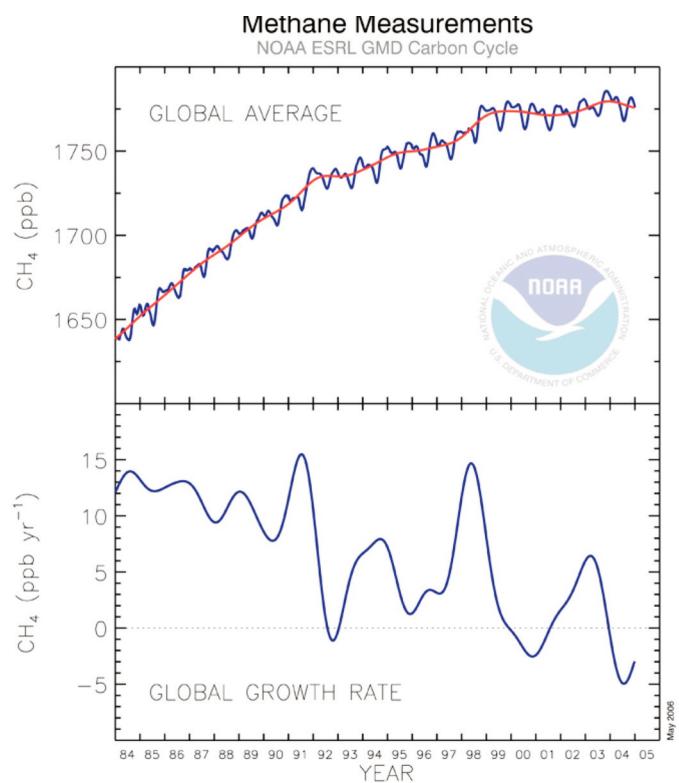
**FIGURA 1.** Evolución de la concentración de dióxido de carbono [CO<sub>2</sub>] en la atmósfera (Fuente: Agencia Europea de Medio Ambiente).

The global CO<sub>2</sub> content in the atmosphere raised from 286 ppm at the beginning of the industrial revolution (around year 1750) up to 388 ppm in 2006 (Canadell *et al.*, 2007), reaching up to 400 ppm in 2015 and 2016 (NOAA, 2016). The [CO<sub>2</sub>] had remained approximately stable with average of 280 ppm during the present interglacial period until year 1750 and at least 10 000 years back. The rate of present increase is not linear and it was accelerated during the last decades. It has been forecasted that the [CO<sub>2</sub>] will reach double the concentration that had before the industrial revolution at the end of this century (Terashima *et al.*, 2014). Figure 1 shows the [CO<sub>2</sub>] evolution since years 1750 to 2010 (European Environment Agency, 2013).

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**METHANE (CH<sub>4</sub>).** Methane is another greenhouse gas with a factor 26 times greater than CO<sub>2</sub> for trapping heat and bouncing it back to the lithosphere. The contribution to the atmosphere is caused by anthropogenic and natural emissions. The anthropogenic causes related to agriculture include mainly ruminant livestock during the processes of digestion and fermentation of cellulose feeds, as well as the CH<sub>4</sub> produced in rice cultivation by artificial flooding irrigation in this crop (Dalsøren *et al.*, 2016). Termites and woodworms, which digest the cellulose of the wood, as well as wetlands and the loss of gas in power generation and mining, also have a remarkable contribution to the emissions of this gas. The atmosphere methane concentration evolved from 1640 ppb in 1984 to 1775 ppb in 2005, according to Figure 2 (NOAA, 2006). The measurement of CH<sub>4</sub> continues increasing and reached 1820 ppb in 2013.

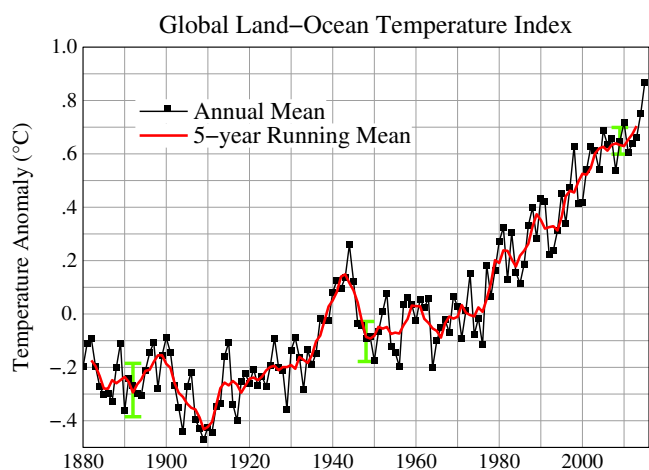


Top: Global average atmospheric methane mixing ratios (blue line) determined using measurements from the GMD cooperative air sampling network. The red line represents the long-term trend. Bottom: Global average growth rate for methane. Contact: Dr. Ed Dlugokencky, NOAA ESRL GMD Carbon Cycle, Boulder, Colorado, (303) 497-6228 (ed.dlugokencky@noaa.gov, <http://www.cmdl.noaa.gov/ccgg>).

**FIGURE 2.** Evolution of methane from 1984 to 2005 [Source: National Oceanic and Atmospheric Administration (NOAA)].

**FIGURA 2.** Evolución del metano desde 1984 a 2005 (Fuente: NOAA).

**TEMPERATURE.** The increases in the content of gases in the atmosphere, such as CO<sub>2</sub>, CH<sub>4</sub>, NO<sub>2</sub> and others, have caused a continuous rise in the global temperature until the year 2015, which was 0.6° C higher than the 20th century average, 0.8° C more than the year 1880 and 1° C greater than the year 1910 (NOAA, 2013; Smith *et al.*, 2008). There is also a high variability in the manifestation of the climate from year to year and from one to another area in the world (e.g., El Niño and La Niña effects).



[http://data.giss.nasa.gov/gistemp/graphs\\_v3/fig.A2.gif](http://data.giss.nasa.gov/gistemp/graphs_v3/fig.A2.gif)  
Update of Hansen, *et al.*, 2010.

**FIGURE 3.** Global change in temperature on earth and the ocean (Datasets from the Hadley Centre HadCM3 Climate Model - See more at:

[http://ete.cet.edu/gcc/?/globaltemp\\_teacherpage/#sthash.Nx8M0ixg.dpuf](http://ete.cet.edu/gcc/?/globaltemp_teacherpage/#sthash.Nx8M0ixg.dpuf)

[http://ete.cet.edu/gcc/?/globaltemp\\_teacherpage/](http://ete.cet.edu/gcc/?/globaltemp_teacherpage/)

**FIGURA 3.** Cambio global de temperatura en la tierra y el océano.

## 2. RELATIONSHIP WITH PLANTS

The present models of biochemical pathways in relation to plant physiology predicted an increase in the rate of plant respiration in the dark, even exponentially, when the temperature increases; however modern empirical research concerning 231 species in 7 biomes has found that the respiration of plants converge at high temperatures due to mechanisms that protect them against higher energy expenditure (Heskel *et al.* 2016). There is not difference in the rate of dark respiration between C3 and C4 plants (Bird *et al.* 1992). In a recent study, when the [CO<sub>2</sub>] increased in artificial atmosphere (from 396 to 792 ppm), there was an increase in transcription of genes that encode enzymes of respiration of plants at the end of the light period, but they did not manifested higher activity (Watanabe *et al.*, 2014). There was also an increase in the production of primary metabolites such as starch and sugars, which denotes a greater photosynthetic activity in a large number of C3 plants, although the studies are still not conclusive in all species. The high [CO<sub>2</sub>] does not increase photosynthetic activity in C4 plants but can indirectly increase C fixation during drought periods in these plants (Leakey *et al.*, 2009). These two factors, increase in [CO<sub>2</sub>] and temperature change of environmental conditions, are both directly related to genes that encode enzymes and proteins of plants that in some cases stimulate their regulation and sometimes decelerate them. A high [CO<sub>2</sub>] increases the C/N ratio by which plants show symptoms of stress as if they had N deficit, with a premature aging of leaves (Terashima *et al.*, 2014). However a high [CO<sub>2</sub>] increases the efficiency in the use of the N and decreases the use of water.

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## 3. GENETIC IMPROVEMENT OF PLANTS

The genetic improvement of plants has been successful in modifying the genotypes of plants to achieve more efficient crops for the desired characters under selection, by using appropriate breeding methods. Plant breeding requires genetic variability in the species that are being improved to reach its objectives. The selection methods evaluate the available genotypes and choose the most suitable for the next generation.

The artificial selection carried out by plant breeders is similar, saving distances, times and scales, to what happens in nature according to the theory of the natural evolution of the species, which was exposed by C. Darwin in his book "On the Origin of the Species" in 1859. The existing variation in biological organisms, which is created by random natural mutations of genes and fortuitous crosses, is subjected to the pressure imposed by the different environmental conditions in the various regions of the world. Only those mutants or favorable genotypes that fit the specific conditions in each environment are capable to survive for the next generation, what determines the evolution and differentiation of the species. Fisher (1930) established the fundamental theorem of natural selection which proposes that the rate of adaptation (fitness) of an organism is proportional to their genetic variation. This was shown later by Falconer and Mackay (1981) and other authors when comparing selection methods in which the genetic gain and the response to selection depends on the genetic variance (Moreno-Gonzalez and Cubero, 1993).

## 4. RECURRENT SELECTION

In all selection schemes, it is necessary to start from a plant material with sufficient and sustained variability throughout the process. One of the most efficient methods is the so-called cyclic or recurrent selection since this has provided good results to improve quantitative characters which are governed by many genes with small effects and segregating at different frequencies in many crops. The selection program aims to gradually increase the frequency of favorable alleles that are responsible for the character of selection, cycle after cycle. The general scheme involves three stages per selection cycle: (1) creation of a family structure; (2) evaluation of families in repeated experiments tested in different environments; (3) recombination of genotypes selected in the evaluation trials (Moreno-Gonzalez and Cubero, 1993). After that, a new cycle starts immediately. This scheme was the base for numerous selection programs, which have been accomplished in public research centers and private seed companies and have achieved an unquestionable success in improving the performance of traits subjected to selection in several grass and forage crops, such as *Zea mays* L., *Lolium perenne* L., *L. multiflorum* L., *Festuca arundinacea* Schreb., *Medicago sativa* L., *Lotus* spp, *Poa pratensis* L., *Trifolium repens* L., *Dactylis glomerata* L., *Secale cereale* L., *Avena sativa* L. and *Eragrostis tef* Zucc.

## 5. KEY EXPERIMENTS OF ARTIFICIAL SELECTION

The most spectacular experiment about artificial selection of plants is the one which is being presently carried out in the University of Illinois. It is a long duration experiment in which selection for both directions high contents in oil and protein and low contents in oil and protein is being conducted in the maize grain. It began in the year 1899 from 163 ears from the maize open pollinated variety called 'Burr White'. The 24 highest in protein ears, the 12 lowest in protein ears, the 24 highest in oil ears, and the 12 lowest in oil ears were selected to start the strains known as IHP ("Illinois high protein"), ILP ("Illinois low protein"), IHO ("Illinois high oil"), and ILO ("Illinois low oil"), respectively (Dudley and Lambert, 1992).

This experiment shows the major genetic changes achieved in quantitative traits, such as protein and oil contents in maize grain for more than 100 generations of selection (Dudley and Lambert, 2004; Moose *et al.*, 2004). Responses to the selection changed in both directions from 4.6% of oil in the original population up to 22.4% in IHO, and down to 0.5% in ILO after 100 generations. Also, populations changed from originally 10.9% of protein up to 30.5% in IHP and down to 4.2% in ILP after 100 generations. Really a maize crop which was initially rich in starch became into an oilseed crop and a protein-rich crop.

Other noteworthy experiment with over 50 years of selection is one carried out in the University of Iowa, where two populations of maize are subjected to the Reciprocal Recurrent Selection method to increase the grain yield. After 11 cycles, the direct response to selection in the cross between the two populations showed an increase of 6.9% per cycle, accumulating 76% improve along the selection process (Keeratinijakal and Lamkey, 1993)

## 6. YIELD IMPROVEMENT OF COMMERCIALIZED CULTIVARS IN DIFFERENT CROPS AND DIFFERENT ERAS

Genetic improvement has helped to raise the yield of many crops over the years, changing its morphological and physiological characteristics at the same time. Some examples are as follows:

**CORN.** Maize yield has been continuously increasing since the adoption of corn hybrids in the 1930's to the present day. Approximately 50% of the increase is due to the genetic improvement and the other 50% to the introduction of modern agronomical and farming practices (Duvick, 2005). However genetic gain will have a greater contribution in the future because there are new tools such as molecular markers to aid in the selection. Not only yield is higher, but also the characteristics of the new hybrids have changed, especially those that confer resistance

to a wide range of biotic stresses, (viruses, pests, diseases) and abiotic stress, such as drought (Campos *et al.* 2006) plant density (Tollenaar, 1989) and lodging tolerances. New hybrids also improved morphological characteristics (e.g., number, deep and size of kernels, upright leaves, greater number of leaves above the ear, stable yield) and physiological changes to promote efficiency of growth and better distribution of metabolites in the plant (e.g., smaller tassels) (Duvick, 2005), as well as better use of nitrogen and water (O' Neill *et al.* 2004). However other characteristics have not changed, e. g., harvest index (Tollenaar and Lee, 2006). Inversely to what occurs in wheat, to get more grain yield, more fodder is needed to synthesize grain starch in the maize plant. Anyhow, the new hybrids yield more than their predecessors in both favorable and unfavorable conditions.

**WHEAT.** The yield of wheat also increased through different eras from the 1930s to the 2000s, as well as the indices of quality of wheat flour. This has been due in large part to the introduction of one, two or more of the dwarf or semidwarf genes (*Rht1*, *Rht2*,..., *Rht14*; *Rht* stands for reduction of height), (Gale and Yousefian, 1985; Ellis *et al.*, 2002; Chen *et al.*, 2013). This has enabled the use of most productive cropping practices, such as application of high nitrogen doses, a higher plant density without increase in plant lodging, as well as an augmentation in the harvest index.

An experiment with 48 varieties of wheat belonging to the above mentioned periods was carried out for the evaluation of the quality of the flour in Turkey. The trials confirmed that there was an increase of the flour protein from 11.34 percent in the varieties of the 1933-1964 period to 12.13 percent in those of the 1991-1996 period; also the modern varieties showed a greater lysine content than the old ones (Anjum *et al.*, 2008).

In Italy, the genetic improvement of wheat achieved a reduction of the sensitivity to photoperiod and an increase of earliness in modern varieties compared with the old ones (Motzo and Giunta, 2007). Also in Spain, the bread wheat yield increased from years 1930 to 2000 at a rate of 35 kg/ha/yr (Sánchez-García *et al.* 2013)

In the North of China, the productivity increased significantly due to the genetic improvement of wheat during the last six decades (1950-2009) (Zheng *et al.*, 2015). In the context of the evaluation of the varieties from different eras, experiments were also carried out in artificial facilities that mimicked a temperature 1.1° C above than that found outdoors. The results showed that the genetic improvement of wheat may have had a positive effect on adaptability of wheat to the impact of global warming (Zheng *et al.*, 2015).

**TOMATO.** A recent study has evaluated a wide range of commercial varieties of tomatoes for industrial processing releasing during the last 80 years (Barrios-Masias and Jackson, 2014). Modern varieties have doubled the yield per hectare relative to the old ones. At the same time, certain phenological characteristics (early



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Harvest of trials of forage maize hybrids.

Recolección de ensayos de evaluación de híbridos de maíz forrajero.

flowering and concentrated fruit juice) were associated with morphological characteristics (smaller plant size and lower biomass), as well as with physiological characteristics (higher concentration of N in foliage and a higher rate of photosynthesis) in modern varieties. These results show that the selection of certain characteristics may be relevant for the future improvement of tomato varieties.

## 7. POLYPLOIDY

It has been widely used as a natural or induced event in many forage species.

**LOLIUM.** Two *Lolium* species widely spread in pastures and meadows, such as perennial ryegrass (*L. perenne*) and Italian ryegrass (*L. multiflorum*) often exhibit autotetraploidy. The autotetraploids are originated by simple duplication of chromosomes in the diploid state. Chromosomal duplication may be artificially induced by application of colchicine in coleoptile or radicle of recently germinated seedlings (i.e., meristematic tissues where intensive cell division occurs), using proper protocols, characterized by chemical doses, temperature, time, and *in vitro* culture media. Some characteristics of the autotetraploid varieties of *L. perenne* and *L. multiflorum* are defined because they have larger cells and leaves than the diploid varieties (Sugiyama, 2005).

Sugiyama's data show that the tetraploid leaves have a greater rate of elongation than diploid leaves, however the duration of the elongation process is not longer in tetraploids. Leaves of tetraploid varieties also reach longest aging, since mature cells have a longer life.

It has been also shown that tetraploid varieties have greater competitive capacity and lower tolerance to cold than diploids. The difference of these characters between the two levels of ploidy is greater than between varieties within the same level. During the process of forming autotetraploids in either *L. multiflorum* or *L. perenne*, no significant differences were found for vegetative characters between euploids and aneuploids in the second and third generation, whereas differences were indeed found for reproductive characters such as seeds, which were favorable for the euploids (Klinga, 1986)

**FESCUE.** The vast majority of commercial varieties of Tall Fescue (*Festuca arundinacea* Schreb. var. *Arundinacea*) growing in Northern Europe and America are alohexaploids ( $2n = 6 \times = 42$ ). Hexaploid means that the basic number of chromosomes, in this case 7, is multiplied by six. The prefix "alo" means that the chromosomes come from at least two different species. In this case the Fescue was originated by crossing two different species. One of them is the diploid Fescue of meadows (*F. pratensis* [Huds.] Darbysh.), which has provided two sets of chromosomes

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designated by PP, while the other species was the tetraploid fescue (*F. arundinacea* var. *Glaucescens* Boiss), which has brought four groups of chromosome designated by G1G1 and G2G2. However the genomic nature of the Mediterranean Fescue, also hexaploid, is different from the Northern Tall Fescue and it has been formed from different diploid and tetraploid species (Oregon State University, 2016).

The *F. arundinacea* Schreb. var. *Arundinacea* has also been called *Lolium arundinaceum* (Schreb.) Darbysh or *Schedonorus arundinaceus* (Schreb.) Dumort (Craven *et al.*, 2009) due to its proximity to the genus *Lolium*. However this change of name has caused much debate among experts and there is not agreement on the subject.

The Tall Fescue is very productive and widely adapted as pasture during cold seasons. It is characterized by having one of the longest periods of growth under cold conditions

**WHITE CLOVER.** Evidence was found recently demonstrating that the white clover (*Trifolium repens* L.) is an allotetraploid ( $2n = 4x = 32$ ) which comes from the crossing between *T. pallenscens* ( $2n = 2x = 16$ ) and *T. occidentale* ( $2n = 2x = 16$ ), (Williams *et al.*, 2012). The *T. occidentale* is predominantly a self-fertile species growing in a habitat restricted to the maritime area near the European coast and “Gulf Stream” influence. In contrast *T. pallenscens* is a clover of Alpine origin, predominantly cross-pollinated, but also self-fertile, with pale pink petals and located preferably in altitudes above 1800 m in Europe. White clover is a stoloniferous plant widely distributed in the grasslands of Europe, Asia and Africa.

Hybridization between *T. pallenscens* as female and *T. occidentale* as male was artificially obtained (Williams *et al.*, 2012). Some of the F1 plants were inter-fertile. Four viable plants were developed from the selection of 200 seedlings obtained in the F1 crossing. One of the plant was the F1 hybrid called 440-1 ( $2n = 16$ ), which incorporated chromosomes of both *T. pallenscens* and *T. occidentale* (Williams *et al.*, 2012) with white flowers. So it is possible to create a wide population of diploid and tetraploid plants originated from that artificial crossing, which incorporates the favorable characteristics of the two *Trifolium* into the white clover genome. This population may be used for selecting new cultivars due to its great variability and plasticity in a future breeding program.

**RED CLOVER.** Red clover (*Trifolium pratense* L.) is an autotetraploide ( $2n = 4x = 48$ ) with induced polyploidy that produces more forage yield than the diploid form, because of the wider leaves due to a larger size of cells especially in the meristematic tissues. They have a longer persistence and higher resistance to the fungus sugar-beet powdery mildew (Jakesova *et al.*, 2011) than the diploids. One problem encountered is that the tetraploid form produces fewer seeds per plant than the diploid. Thus use of commercial tetraploid seed is not economically profitable so far (Vleugles *et al.*, 2015).

## 8. INTERGENERA AND INTERSPECIES HYBRIDIZATION

Hybrids between different species, either from same or different genus, produce in general heterosis, i.e. an offspring which shows higher yield and qualities than the average of the two parents, and in many cases it is superior to the best parent. But above all, it creates a great variability in the population resulting from the crossing, which in turn it allows selecting the best genotypes in each selection cycle and incorporating the favorable characteristics from each one of the parents into the commercial varieties that are being developed.

Hybridization is not always easy. In many cases, it is necessary to perform the emasculation of the female parent and even more complex techniques to overcome the sexual incompatibility barriers that exist between species. It should be studied which parent is the most suitable to use as a female concerning the yield of viable seed. Thus reciprocal crossings need to be evaluated by exchanging the parental male and female. Another problem arising in interspecies hybridization is that the seed from the cross is not always fertile or is only partially fertile due to unbalance in the number of chromosomes (aneuploidy). In these cases, it is possible to use the immature embryo rescue technique by transferring the embryos to *in vitro* culture for their development according to suitable protocols of favorable conditions, such as temperatures, enzymes, chemical components and time. Selection among aneuploid plants from F<sub>1</sub>, F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>... generations of inter-species hybrids for achieving balanced euploid plants is needed for getting stable commercial hybrids. Some examples of interspecies hybridization are as follows:

**HYBRID OF *L. MULTIFLORUM* × *L. PERENNE*.** The cross between perennial ryegrass (*L. perenne*) × Italian ryegrass (*L. multiflorum*) is an allotetraploid ( $2n = 4x = 28$ ). It can be produced artificially, but it sometimes arises naturally. This hybrid incorporates the permanence of the perennial ryegrass in the field and the fast growth of the Italian ryegrass, in addition to the heterosis vigor. It is a fast growing species, which combines semi-prostrated and erect stems providing versatility for consumption in grazing and cutting.

A study of chromosome association comparing the allotetraploide hybrid (*L. perenne* × *L. multiflorum*) with the autotetraploide of perennial ryegrass (*L. perenne*,  $2n = 4x$ ) showed that the average ratio between the number of tetravalent matings / number of bivalent matings was 0.18 for the allotetraploid hybrid while it was 0.49 for the autotetraploide (Deniz and Dogru, 2008). This indicates that the allotetraploid hybrid population must be further selected for increasing the number of tetravalents through successive generations to reach a stable hybrid population where individual plants can produce viable seeds. The stable hybrid population will become a potential breeding material useful for the development of new hybrid cultivars.

**HYBRID OF *L. MULTIFLORUM* × *L. TEMULENTUM*.** The cross between a variety of *L. multiflorum* and another of *L. temulentum* originated self-fertile F1 plants capable of producing F2 seeds with about a 57% germination rate when temperature conditions were adequate (Kiyoshi *et al.*, 2012). This indicates the possibility of producing recombinant lines between the genomes *L. temulentum* and *L. multiflorum* and selecting among them the most suitable genotypes.

**FESTULOLIUM** is called the cross between a *Festuca* spp. and a *Lolium* spp. Many possible combinations between these two genera were found. In general, two species of fescue, *F. arundinacea* and *F. pratensis* may cross each one by two raygrasses, *L. perennial* and *L. multiflorum* giving rise to four Festulolium. The proximity of *Festuca arundinacea* to the genus *Lolium* is so great that the first species has been recently named *Lolium arundinaceum* although later evidence was found for not accepting this change of name. The four Festulolium hybrids are as follows:

Maternal Progenitor	Paternal Progenitor	Progeny of Hybrid
<i>Festuca arundinacea</i>	<i>Lolium multiflorum</i>	<i>Festulolium pabulare</i>
<i>Festuca arundinacea</i>	<i>Lolium perenne</i>	<i>Festulolium holmbergii</i>
<i>Festuca pratensis</i>	<i>Lolium multiflorum</i>	<i>Festulolium braunii</i>
<i>Festuca pratensis</i>	<i>Lolium perenne</i>	<i>Festulolium loliaceum</i>

The two last Festulolium are allotetraploids of chromosomal constitution ( $2n = 4x = 28$ ) according to the following nomenclature:

*Festuca pratensis* ( $2n = 2x = 14$ ) × *L. multiflorum* ( $2n = 2x = 14$ ) = *[Festulolium braunii* ( $2n = 4x = 28$ )]

*Festuca pratensis* ( $2n = 2x = 14$ ) × *Lolium perenne* ( $2n = 2x = 14$ ) = *[Festulolium loliaceum* ( $2n = 4x = 28$ )]

The Festulolium which is close to the fescue type can be characterized by: plant vigor similar to Fescue; spring growth earlier than Fescue; high performance; high-quality forage similar to rye-grass; tendency to bloom only in the 1st cut; high persistence; growth upwards; tolerance to drought and floods; resistance to winter cold. Other species of Festulolium have been named according to the following nomenclature (Hopkins *et al.*, 2009) (table 1).

**TABLE 1.** Nomenclature of hybrid species of Festulolium (Hopkins *et al.*, 2009).

**TABLA 1.** Nomenclatura de especies híbridas de Festulolium (Hopkins *et al.*, 2009).

Parental femenino	Parental masculino	Especie híbrida
<i>F. arundinacea</i>	<i>L. multiflorum</i>	<i>Festulolium pabulare</i>
<i>F. arundinacea</i>	<i>L. perenne</i>	<i>Festulolium holmbergii</i>
<i>F. gigantea</i>	<i>L. perenne</i>	<i>Festulolium brinkmannii</i>
<i>F. gigantea</i>	<i>L. multiflorum</i>	<i>Festulolium nilssonii</i>
<i>F. pratensis</i>	<i>L. multiflorum</i>	<i>Festulolium braunii</i>
<i>F. pratensis</i>	<i>L. perenne</i>	<i>Festulolium loliaceum</i>
<i>F. pratensis</i>	<i>L. perenne</i>	<i>Festulolium elongata</i>
<i>F. pratensis</i>	<i>L. perenne</i>	<i>Festulolium loliacea</i>
<i>F. rubra</i>	<i>L. perenne</i>	<i>Festulolium frederici</i>

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Seed multiplication field of Italian Ryegrass (*Lolium multiflorum* Lam. cv. Pomba) registered by CIAM

Campo de multiplicación de semillas de Raigrás Italiano (*Lolium multiflorum* Lam. cv. Pomba) registrado por el CIAM.

Many varieties of Festulolium have been already registered in the national list of varieties in different countries of Europe (Ghesquiere *et al.*, 2010)

**BLUEGRASS HYBRIDS (BGH)** were developed by crossing two species, Texas bluegrass (TBG) (*Poa arachnifera* Torr.) × Kentucky bluegrass (KBG) (*Poa pratensis* L.). Studies of comparisons of BGH cultivars with KBG cultivars suggested that the BGH exhibited improvement in drought stress, heat tolerance and resistance to the fall armyworm *Spodoptera frugiperda* (J. E. Smith) (Lepidoptera: Noctuidae), relative to KBG (Merewitz, 2011; Reinert and Read, 2008). The maintenance of higher rates of transpiration and photosynthesis, water use efficiency (WUE) and increased root viability was associated with the improvement in heat tolerance of the BGH cultivars (Abraham *et al.*, 2008). In another study, BGH cultivars revealed significantly ( $P < 0.05$ ) higher turf color indices and lower water-use in comparison with Tall Fescue cultivars (Githinji *et al.*, 2009). Likewise, intra-specific hybrids of Kentucky bluegrass (*Poa pratensis* L.) are also developed from the cross of two elite bluegrass cultivars. After evaluation of seed-propagated plants that are harvested from single-clone apomictic genotypes in the F<sub>1</sub> generation, the best intra-specific hybrids are selected and registered as new cultivars for commercialization. Some of them are claimed to show tolerance to low mowing heights and resistance to some important turf diseases (Brede, 2011).

## 9. SYNTHETIC VARIETIES

The most efficient way of exploiting heterosis in cross-pollinated plants is through the development of hybrids. However, hybrid seeds are only used commercially if they are easy to produce and if the advantages offered by the best performance of hybrids will compensate the high expenses incurred

1	9	5	10	4	8	2	6	3	5
2	10	6	5	1	3	7	8	9	4
3	8	7	6	7	9	5	10	1	2
4	2	10	1	8	5	6	3	10	7
5	3	8	9	6	3	4	1	2	9
6	1	9	4	10	7	3	2	7	8
7	4	1	2	3	10	9	5	8	6
8	5	3	4	2	6	1	7	9	10
9	7	4	8	5	1	10	6	4	3
10	6	2	7	9	2	8	4	5	1

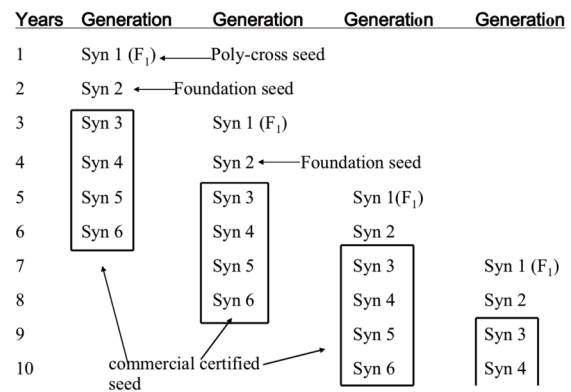
**FIGURE 4.** Scheme of polycrosses between selected clones (generation Syn0) for harvesting seed of the generation F1 of a synthetic variety (Syn 1).

**FIGURA 4.** Esquema de policruzamientos (generación Syn 0) entre clones seleccionados para cosechar semilla de la primera generación F1 de una variedad sintética (Syn 1).

as a result of hybrid seed production. In other cases, the development of synthetic varieties (SV) is the best option. The breeding procedure for development of SV consists in making crosses among elite plant clones according to a partial diallel design, following by evaluation of their progenies in replicated trials in various environments and years and selecting the clones that have shown better general combining ability (GCA). Once the best clones are selected, they should be laid out according to a scheme of poly-cross (similar to a *sudoku*) called generation Syn 0, in such a way that each clone receives pollen from the others. The harvested seed constitutes the first generation Syn 1 of the SV (Figure 4).

The Syn 1 is an F<sub>1</sub> generation mixture of seed harvested in each clone. According to genetic theory the F<sub>1</sub> performance is generally superior to the F<sub>2</sub> and successive generations. The Syn 2 is obtained from Syn 1 by open-pollination of bees and wind and it is called foundation seed; its performance is equivalent to an F<sub>2</sub> generation and remains stable for the remaining generations. Seed companies receive the foundation seed from breeders and multiply it by open-pollination for three or four further generations to increase the amount of commercial seed of the synthetic varieties during different generations, which are called Syn 3, Syn 4, Syn 5, and Syn 6 (Figure 5). All these generations Syn 2 to Syn 6 are equivalent to and F<sub>2</sub> and are certified seeds to be used by farmers. After the 6th generation, it is considered that the seed may have lost its purity and it is necessary to go back to reconstitute the Syn 1 with the original clones that remain in the poly-cross field of breeders, and the process continues. The commercial SV are used mostly for cross-pollinated grasses and forage species

**ALFALFA.** Evolution of alfalfa yield in the field along years shows discrepancies according to the different authors consulted. Comparison of trials of alfalfa varieties released in different “Eras” shows that there was genetic gain for yield between Era 1 (1898-1900) and Era 2 (1940-1950), but not between Era 2



**FIGURE 5.** Multiplication of F<sub>1</sub>, foundation seed and certified seed of synthetic varieties along different years and generations.

**FIGURA 5.** Multiplicación de F<sub>1</sub>, semilla base y semilla certificada de variedades sintéticas durante varios años y generaciones.

and Era 3 (1975-1980), resulting in a stagnation from 1955 until 1975, although varieties of Era 3 were most enduring in the field than those of Era 2 and these in turn were even more than those of Era 1 (Holland and Bingham, 1994). In other studies of trials in different universities of USA, there was no evidence to conclude that performance had changed over the decades prior to 1977 (Wiersma *et al.*, 1977). According to crop annual statistics from the USDA no increase in alfalfa yield was noted in farmer fields from 1919 until 1955, although there was increased in the period from 1955 until mid- to -late 70s. After this date there has been stagnation. However, a study conducted by Loiselle (1992) indicates an increase in yield of 1% between 1975 and 1992. Several studies reported on the contribution of breeding to alfalfa yield with a genetic yield gains of 0.15-0.3% per year since the late 1950s up to mid 1980s and early 1990s (Hill *et al.*, 1988; Holland and Bingham, 1994).

In general, most of the studies conclude that the genetic gain in alfalfa varieties through different eras was low until 1990.

## 10. INTRA-SPECIFIC HYBRIDS

**MAIZE.** The increase in size and vigor of hybrids between varieties and races of different origin has been known for several centuries and the old breeders had used it empirically to develop new cultivars. The first hybrids were described by Shull (1908), which marks the beginning of the exploitation of heterosis in maize. Since then, and mainly after 1930, the surface of maize sown with hybrid seed have augmented first in USA and then in Europe and around the world (Hallauer *et al.* 1988). Maize yield also continues increasing and today the challenge of seed companies is to develop new corn hybrids that provide greater performance than those released in the previous years. Additionally, new hybrids continue to be more resistant to pests, diseases and abiotic stress than old ones. The superior performance of newer hybrids at high density reveals a better tolerance to stress in these hybrids (Chen *et al.* 2016).

The varieties of maize crop can be adapted to environmental conditions different from those where they were originally grown by using proper breeding methods. This is the key of success why high-yielding maize varieties have spread all over the world in relative short time. One example is the elite maize Hybrid B73 x Mo17 developed by Iowa State University, USA, in the 1970s. Hybrids related to B73 x Mo17 were sown worldwide and are still being cultivated in large part of the world today. Variability in the maize genome, which is conserved in germoplasm banks, is very large for many traits, including those related to adaptability, such as growing degree units (GDU) up to flowering (Rincen *et al.* 2014), GDU up to harvesting, cold tolerance (Revilla *et al.*, 2014, 2016), drought tolerance and resistance to diseases and pests. This variability for several traits may be incorporated into lines B73 and Mo17 to obtain lines related to B73 and Mo17, which still continue showing heterosis. Adaptation of B73 and Mo17 to shorter season environments has been carried out in the Center of Agriculture Research at Mabegondo (CIAM), A Coruña, Spain. More than 120 lines were developed from backcrosses and crosses of B73 to early germplasm material. All 120 lines were crossed to the tester EC560, which is related to Mo17, and compared to the reference Hybrid B73 x Mo17, in addition to three commercial checks, and the cross of line B73 to tester EC560 in replicated trials in three environments. The results of hybrids of the top-20 selected lines crossed by a tester concerning biomass dry matter and growing degree units (GDU), which is the sum of average daily temperature above 6 °C threshold and expressed in °C d, from planting up to female flowering are presented in Table 2. All crosses were earlier than the reference hybrid B73 x Mo17 as indicated by lower GDU for females flowering. In addition some of the crosses yielded equal or higher than the reference hybrid. Trying to look for early hybrids, the EC611 x tester EC560 seems interesting because it was 120 GDU less, approximately 9 days earlier at female flowering time, it also had 8% more dry matter at harvest (DMH), lodged 2% less, and only yielded 2 t/ha less than the B73 x Mo17 reference hybrid (data not shown for DMH and lodging).

The example of corn has moved to the development of hybrids in other cross-fertilized crops, such as sunflower, sugar beet, cabbage, *Brassica rapa* (Chinese cabbage), rapeseed, rye, and in some fertilized crops, such as tomato, wheat etc, in addition to pasture cops. Hybrids are generally higher yielding than varieties.

**PASTURE LEGUME CROPS.** The development of semi-hybrids and hybrids has been also proposed in clovers and alfalfa.

**CLOVERS.** An example is the development of the semi-hybrids which has been proposed in red clover. The method is to restrict the self-incompatibility by using three self-incompatible alleles in the population “A”, such as S1, S2 and S3. This population is pollinated by bees with pollen from another population “B” that shows heterosis with A and has a S0 allele cross-compatible with the previous ones in order to form the hybrid A x B. All plants with

**TABLE 2.** Best linear unbiased estimate (BLUE) means of hybrids of the top-20 selected inbred lines developed from material involving B73, and crossed to tester EC560 related to Mo17 over three environments for forage dry matter (DM) yield and growing degree units (GDU) up to female flowering.

**TABLA 2.** Medias BLUE de híbridos de las 20 mejores líneas dentadas seleccionadas a partir de material relacionado con B73 y cruzadas por el tester “EC560” relacionado con Mo17, evaluados en tres ambientes para el rendimiento de materia seca del forraje y los grados-días hasta la floración femenina.

Pedigree of lines ‡	Inbred lines	Forage DM Yield (t/ha)	Female GDU (° C d)
EC136_B73(2)-1-1	EC612A, B	23.31	933.83
EC134_B73(2)-5-1	EC619A, B	23.40	986.88
EC133A_B73-7-1-1	EC605	22.40	933.12
EC135(2)_B73-3-2	EC622A	21.94	954.46
<b>B73 x Mo17 †</b>	Reference1	22.04	1010.84
EC134_B73(2)-7-1	EC620, EC621A-D	21.41	953.26
EC135_B73(2)-6-1	EC625	21.07	978.34
EC136_B73-9-1-1	EC611	20.05	891.73
EC133A_B73-4-1-2	EC604	21.16	940.09
<b>B73 x EC560 †</b>	Reference2	21.16	1028.30
EC134_B73(2)-3-1	EC627	20.98	989.93
EC134_B73-2-1-1	EC618	20.54	947.58
EC136_B73-4-1-2	EC628	20.27	915.13
EC135_B73(2)-2-1	EC629	20.15	968.20
EC134_B73(2)-6-1	EC630	20.63	995.07
EC133A_B73(2)-1-3	EC606	20.26	972.01
EC136(2)_B73-4-1	EC609A, B	19.38	906.22
EC136_B73-1-1-2	EC610A, B	19.10	920.30
<b>Experimental Hybrid</b>		19.79	935.91
EC135_B73-8-1-1	EC624	19.71	961.03
<b>DKC4117 (check1)</b>	Commercial1	18.87	877.98
EC133A(2)_B73-1-1	EC601	18.97	955.09
EC133A_B73-8-1-1	EC632	18.48	939.58
EC133A(2)_B73-3-3	EC602	18.86	956.33
<b>LG3303 (check 2)</b>	Commercial2	16.41	845.43
<b>ELDORA (check 3)</b>	Commercial3	16.79	1021.35
Standard error		1.13	25.20

† Reference hybrids B73 x Mo17 and B73 x EC560;

‡ All lines were crossed to tester EC560 related to Mo17, except commercial checks

sporophyte of genetic constitution S1S2, S1S3 and S2S3 from population “A” can receive pollen carrying the allele S0 from the “B” population or any other allele that is not part of the sporophyte, in this case S3, S2 and S1 allele, respectively. This gives rise to semi-hybrids (Friday & Krohn, 2010).

**ALFALFA.** The great jump for developing hybrid varieties of alfalfa was the finding of a male-sterile cytoplasm (MSC) (Pedersen and Stucker, 1969; Brown and Bingham 1984). An *Rf* gene that restores fertility in the MSC was also found, what allows developing MSC lines. A patent exists on this subject for hybrid seed production. The female parental line, say A(MSC, *rfrf*) has male-sterile cytoplasm and *rfrf* genes, which do not restore fertility. The maintainer line, say A(N, *rfrf*) is used as male to multiply the line A(MSC, *rfrf*). The male maintainer has normal cytoplasm (N); thus, it is fertile and has the same genotype as A(MSC, *rfrf*) for the great majority of all remaining genes. The cross A(MSC, *rfrf*) x A(N, *rfrf*) will be able to produce enough seed of A(MSC, *rfrf*) to be used as female parental in the hybrid production field.

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Seed multiplication field of Red Clover (*Trifolium pratense* L. cv. Maragato) registered by INIA-CIAM.

Campo de multiplicación de semillas de Trébol violeta (*Trifolium pratense* L. cv. Maragato) registrado por el INIA-CIAM.

Another line, say B(N, *rfrf*) that shows heterosis with A should be found. Simultaneously, the restorer *RfRf* genes of fertility are introduced in B by recurrent backcrosses of B(N, *rfrf*) to a donor line carrying restorer genes, say Y(N, *RfRf*). This produces the male line B(N, *RfRf*) with normal cytoplasm. The cross A(MSC, *rfrf*) × B(N, *RfRf*) produces the hybrid [A × B](MSC, *RfRf*) which is fertile. The hybrid seed production is sketched in Figure 6.

The process of obtaining all required lines, which are involved in hybrid production, is laborious. Line A(MSC, *rfrf*) is previously developed by repeated backcrossing of A(N, *rfrf*) to a source line having MS cytoplasm, say X(MSC, *rfrf*). Simultaneously, line B(N, *RfRf*) is created by the same method. In addition lines A(N, *rfrf*), B(N, *rfrf*) should be also conserved. (Figure 6)

One of the first commercial hybrid alfalfa variety was tested in 25 trials together with other commercial and experimental no-hybrid varieties through six US Central States during years 1998 to 2001 (Wiersma, 2001). Consistently, the hybrid alfalfa ranked in the top 10% of each test environment; meanwhile the next most consistent no-hybrid variety only had four appearances in this category. This gives an idea of the stability of the hybrid variety though environments (Wiersma, 2001).

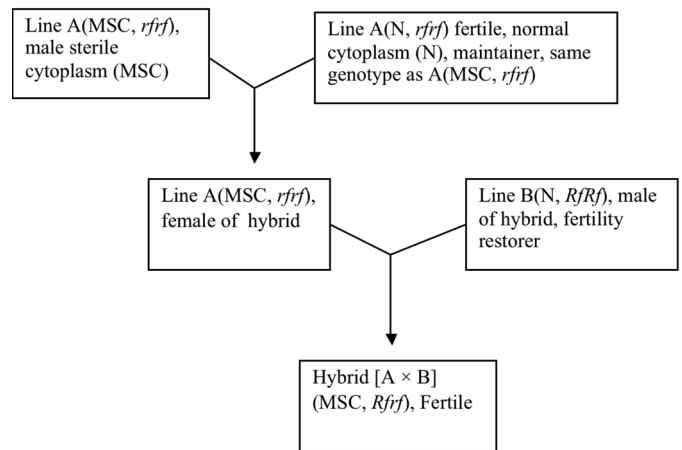


FIGURE 6. Scheme for the production of an alfalfa hybrid.

FIGURA 6. Esquema de producción de un híbrido de alfalfa.

### 11. MOLECULAR MARKERS

A large influx of scientific data has been accumulated over the past thirty years, demonstrating the utility of molecular markers (isoenzymes, RAPDS, RFLPs, SSRs, AFLPs, and SNPs) to examine and map the identity of the genotypes of different varieties of corn and other crops (Senior et al., 1998). Molecular markers have also confirmed its utility to aid in the selection and the development of new varieties, what is called

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marker-assisted selection (Ribaut and Hoisington, 1998; Moreno-Gonzalez, 1999; Chang *et al.*, 2016). High density markers, specially SNPs, along the genome allows to determine the placement of genes and the position of QTLs associated to traits of interest (Rincent *et al.*, 2014).

In addition markers have been proposed to establish genetic variability and distances among populations of both alfalfa and clover from different regions of the world with the purpose of searching for heterosis in improvement programs, what may be used for creating hybrids and semi-hybrids (Nagl, 2011). As the genetic distance is higher, it would be expected greater heterosis.

## 12. C4 PLANTS

C4 plants are named so because the first product of carbon fixation in photosynthesis is a molecule of four carbons, the oxaloacetic acid (OAA), followed by the formation of a four-carbon organic compound, malate (M). Biochemical pathway reactions that occur in the mesophyll and bundle sheath cells of C4 plants were first described by Slack and Hatch (1967). More detailed C4 pathways were later studied (Gutierrez *et al.* 1974). In addition, Sage (2003) studied the evolution of plants and first appearance of the C4 pathway. He estimated that the earliest C4 photosynthesis plants arose in grasses, probably 24–35 million years ago. The earliest C4 dicots are likely members of the Chenopodiaceae dating back 15–21 million years; however, most C4 dicot families are estimated to have appeared relatively recently, perhaps less than 5 million years ago (Sage, 2003).

In contrast, the C3 plants originate as the first product of photosynthesis, a three carbon compound, the phosphoglycerate.

The C4 plants are more efficient than the C3 in the fixation of CO<sub>2</sub>, especially in conditions of drought and heat, since they use the CO<sub>2</sub> from photorespiration, even when the stomata are closed because of drought and hot. In order to not miss the CO<sub>2</sub> produced in the photorespiration and to encourage the binding of this molecule to the Rubisco enzyme, the C4 plants have modified the anatomy of their plant tissue cells. Vascular bundles (phloem) of leaves are surrounded by two layers of cells. The inner cells have chloroplasts where photosynthesis occurs, while the outer cells have low conductance to the diffusion of CO<sub>2</sub> due to a suberization of their cell walls, thus preventing the exit of the CO<sub>2</sub> of photorespiration.

The C4 plants also use the N more efficiently. Only 3.2% of the total number of species in nature, about 8100, are C4 (Sage, 2017). However, the C4/C3 distribution predicts the global coverage of C4 vegetation to be 18.8 million km<sup>2</sup> (17.7 % of total), while C3 vegetation covers 87.4 million km<sup>2</sup> (82.3 % of total) (Still *et al.*, 2003). These authors estimated that the gross primary production (GPP) of C4 plants is 35.3 Pg

C/ year, about 23.5 % of total GPP, while that of C3 plants is 114.7 Pg C/year, about 76.5 % of total GPP (Still *et al.*, 2003). With different simulation model, Ito (2003) estimated that 20.3% of total leaf mass was attributable to C4 plants, besides that 8.7% of soil organic matter is due to C4-derived carbohydrates. Accordingly, C4 plants may play a quantitatively important role in the global carbon cycle.

The C4 crops include maize (*Zea mays*), sorghum (*Sorghum bicolor*), sugarcane (*Sacharum officinalis*), millet (several species), Bermuda grass (*Cynodon dactylon*) used as turf grass in hot environments, switchgrass (*Panicum virgatum*) which has been utilized as a source of biofuel, purple nutsedge (*Cyperus rotundus*), johnsongrass (*Sorghum halepense*) in addition to some amarantaceae and chenopodiaceae. A C4 crop that has attracted attention in recent years is “Teff” (*Eragrostis teff*) from Ethiopia that has been recently spread over arid areas of the world and has also been tested in Spain and may be used for grain and forage production. The teff yield response to irrigation has been simulated in semi-arid environments by using the FAO “AquaCrop” model, calibrated with data from the North of Ethiopia (Araya *et al.*, 2010). The model revealed that the water use efficiency (WUE) for teff grain increased when a supplemental irrigation from 0 to 95 mm was applied after flowering; however the WUE for teff biomass decreased when the same irrigation was applied (Araya *et al.*, 2010). Thus, the biomass crop is more resistance to drought than the grain crop. It is suggested that other C4 plants like Teff, including semi-domesticated forage species, should be explored to find tolerance to drought.

Another interesting area of research would be to explore the possibility of converting a plant C3 to C4, likely using genetic engineering tools; however achieving this goal is not an easy task since it implies changing many genes responsible for the anatomy of plant tissues, as well as for the biochemical pathways of photosynthesis. There is a research proposal in rice that tries to discover the genes responsible for the C4 biochemical processes and the anatomy of arrangement of cells in leaf tissues (Zhu *et al.*, 2010; Glover, 2014). The crossing of a C4 by a C3 plant that would be capable of producing viable seed would open routes to this research.

## 13. C3 PLANTS

### Discrimination of the <sup>13</sup>C isotope in C3 plants under drought. Water use efficiency (WUE)

One of the challenges necessary to be answered will be modeling the adaptation of plants to the undeniable increase in temperature. Higher temperatures will cause more evapotranspiration in plants to maintain the thermal equilibrium necessary for preserving efficiently biochemical reactions in



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Seed multiplication of Tall Fescue (*Festuca arundinacea* Schreb.) in a winter house isolated small chamber. Entry from the germplasm bank of CIAM.

Multiplicación de semilla de Festuca Alta (*Festuca arundinacea* Schreb.) en cámara pequeña aislada de invernadero. Entrada del banco de germoplasma del CIAM.

leaves, and therefore a greater expenditure of water will be necessary. Leaf temperature was negatively correlated with transpiration because of stomata close, air vapor pressure deficit (VPD), and soil water (Pallas *et al.*, 1967). This is undoubtedly an imminent scenario to which crops will have to adapt. Thus, it seems imperative to select plants for greater WUE.

The  $\Delta^{13}C$  is mainly due to lower reactivity of the  $^{13}C$  isotope for the carboxylase enzyme, ribulose-1, 5-bisphosphate (RuP2) compared to the  $^{12}C$  isotope. Another reason for discrimination is due to higher resistance to the diffusion of the  $^{13}C$  with respect to the  $^{12}C$  when  $CO_2$  enters the plant through the stomata opening (Farquhar *et al.*, 1982).

Superior C3 plants which possess the conventional biochemical route of assimilation of carbon have a ratio (R) of isotopes  $^{13}C/^{12}C$  between 20 and 38 ‰ lower in the plant tissues than in the outside atmosphere, whereas in plants C4 which use the route of the dicarboxylic acid, the ratio R is between 10 and 20 ‰ lower in the plant tissues than in the atmosphere, with wide variation in measurements from some authors to others (Bender, 1968; Bender, 1971; Farquhar *et al.*, 1982). The isotope discrimination of  $^{13}C$  ( $\Delta^{13}C$ ) is defined as a simple mathematical formula between the R of any product after the carboxylation in the plant ( $R_{product}$ ) and the R in the atmospheric air ( $R_{air}$ ) (Farquhar *et al.* 1982), according to the following equation:

Regardless of the mechanism operating in the assimilation of carbon, a canopy that uses more water than another will have more open stomata causing a drop in leaf temperature and a lower discrimination of  $^{13}C$  ( $\Delta^{13}C$ ) in the plant. Therefore the parameter  $\Delta^{13}C$  seems relevant to select in hot environments under irrigation (Araus *et al.*, 2002). On the other hand, when additional water is not available for cultivation, WUE seems to be an alternative strategy to improve the performance of the crop. In this context the parameter  $\Delta^{13}C$  is a simple but reliable measure of the WUE. In contrast to the wheat varieties that yield more grain due to higher access to water, the wheat varieties that produce more biomass in relation to a better WUE will have a less  $\Delta^{13}C$ . The WUE can be modified not only by a decrease in the conductance of the stomata, but

$$\Delta^{13}C (\text{‰}) = 1000 \times (1 - R_{product} / R_{air})$$

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also by an increase in the photosynthetic capacity of the plants (Araus *et al.*, 2002). In the case of rice under water stress conditions, the  $\Delta^{13}\text{C}$  of leaves and straw were positively correlated with both grain yielding and WUE genotypes (Akhter *et al.*, 2010).

## 14. FUTURE ORIENTATION OF THE GENETIC IMPROVEMENT FOR MITIGATING THE CLIMATIC CHANGE

**Climate.** The climatic change causing a rise in temperature and an increase in the concentration of  $\text{CO}_2$  in the atmosphere, according to current data, is expected to increase climate variations among different years and different regions (Rosenberg *et al.* 2003). This may cause an increase in the occurrence of abiotic stress such as heat, drought and flooding, which in turn may trigger phenomena of more rain, fluctuating with phenomena of more drought. The associated effects of climate and higher concentrations of  $\text{CO}_2$  include impacts on the water use efficiency (WUE) in both non-irrigated and irrigated land crops, as well as on bio-safety production and quality of products, in addition to impacts on introduced and endemic pests and diseases (Chapman *et al.*, 2012). However, there is uncertainty about the direction and magnitude of changes in the regime of flooding and drought in different parts of the world according to the climate model scenarios. For example, in a scenario with a plausible global warming increase in  $1.9^\circ\text{C}$ , about 1/6 of world human population would be exposed to higher water stress around 1/15 would be threatened with increased river flooding, and 1/5 of world people would be flooded by coastal floods each year (Arnell *et al.* 2016). Under the same scenario, crop productivity would fall in most regions, and home energy demands would be reduced in most areas because lesser heating demands would largely offset the higher cooling demands.

In other study, the analysis of projections of future floods and droughts in Europe and Spain using climate and hydrological simulation models showed that the impact of  $+2^\circ\text{C}$  global warming will be most extreme for France, Spain, Portugal, Ireland, Greece and Albania (Roudier *et al.* 2016). These results were particularly robust in southern France and northern Spain

**CROP ADAPTATION AND BREEDING.** The adaptation of crops to the climatic changes should be faced from several points of view (Chapman *et al.*, 2012). In the short term, the adaptation includes changes in crop management, farm structure and product transformation chain. Sustainable techniques concerning machinery, soil tillage, efficient water management, and rational use of fertilizers and phytochemicals should be adopted for obtaining competitive products and higher added value for farmers and consumers.

In the long run, plant breeding should be the best choice for developing new varieties with tolerance to the weather challenges. The analysis of abiotic stress in all its facets is important

to describe the environments in which new genotypes should be selected. High-throughput phenotyping platforms are now being used to capture and analyze multi-data for bridging the gap between phenotyping and genotyping, and for mimicking the growth plant at different scales and environments, including stress environments. A set of articles on the current state of art in plant phenotyping was published in the special issue of the journal *Functional Plant Biology*, 2017, 44 (Hawkesford and Lorence, 2017). Automated robotic field phenotyping platforms have been installed in some research centers, like the one existing in Rothamsted Research, Harpenden, Herts, UK (Viret *et al.*, 2017).

The future environments will be conditioned by higher temperature, higher  $\text{CO}_2$  concentrations, and drought and flooding episodes (Arnell *et al.* 2016; Roudier *et al.* 2016). The increase in temperature will cause greater evapotranspiration from the plant canopy, as well as greater evaporation from the oceans, seas and lakes. This will affect to an increased movement of air jet streams in the atmosphere through a higher imbalance between low and high pressure areas, as well as in the ocean streams, (example of El Niño and La Niña). In relation to the biotic stresses, it will be necessary to avoid catastrophic situations due to a higher incidence of pests, virus and diseases. Much more research needs to be done to prepare for an era of increasingly severe virus epidemics and increasing difficulties in controlling them, so as to mitigate their detrimental effects on future global food security and plant biodiversity (Jones, 2016).

Assessment of the genetic variability available in the cultivated species, as well as its characterization is essential for incorporating selectively the useful genotypes into the breeding material. The recurrent selection is the best option to achieve the demanded varieties, through evaluation of genotypes and recombination of the selected individuals, cycle after cycle of selection. After several cycles of selection presumably, it will be obtained varieties adapted to the environment defined by the future production systems and the weather conditions.

Plant breeding technologies based on biotechnology, such as genetic modification and marker-assisted selection are essential to accelerate genetic gain, but the application requires additional investment in understanding genetic and phenotypic characterization of complex traits adapted to the new climatic conditions (Chapman *et al.*, 2012).

**EFFECTS OF TEMPERATURE, WATER AND  $\text{CO}_2$ .** The direct effect of temperature on plants will affect the cycles of the phenological stages of C3 and C4 plants, which are manifested by the seed germination, the growth period, the flowering time and the ripening or harvesting season. Water consumption will increase in C3 plants, but also moderately in C4 plants.

The highest concentration of  $\text{CO}_2$  will produce favorable effects, although relatively small, on the photosynthetic activity of C3 plants as well as very small effects on the C4 plants although only limited for drought conditions. The stimulation of yield by

elevated CO<sub>2</sub> in crop species is much smaller than expected (Leakey *et al.* 2009). The extensive literature on the impacts of climate change demonstrates both the positive and negative effects of rising CO<sub>2</sub> on photosynthesis in C3 and C4 plants (Reddy *et al.* 2010). The interaction of increasing CO<sub>2</sub> with other environmental factors, such as water availability, temperature and mineral nutrition should be better studied for understanding the responsiveness of plants to elevated CO<sub>2</sub> atmosphere.

**DOMESTICATION OF CANDIDATE WILD SPECIES.** Domestication of wild species may be another alternative for facing the environmental change. There has been also much effort trying to domesticate wild species with unequal success in achieving new crops for specific environments. For example, the development of perennial grain crops would be interesting from two points of view high biomass production and sustainable production on marginal land. Breeding programs aimed at domesticating wild species have been frequently disappointing. However, a growing body of evidence is demonstrating that the breeding of perennial grains is both necessary and feasible; even when the process may last several decades and must overcome or circumvent genetic complications (Cox *et al.* 2006).

A new approach is proposed suggesting rational steps to attain the domestication goals of grain plants (DeHaan *et al.* 2016) and by extension to other crops, such as horticultural, ornamental and pasture. DeHaan *et al.* (2016) discuss in their article the procedures to get domestication in an efficient way regarding research investment of economical resources, time for getting the objectives as short as possible, and added value of the domesticated crop products.

The plant domestication efforts should be carried out to meet a particular need or solve a problem in consecutive phases. It is important to define the agricultural context for which plants will be growing; e.g. temperature, rainfall distribution and amount, as well as salinity conditions of future environments. Then, various species should be evaluated to eventually develop at least one new crop that meets the requirements. The first screening process is based on consulting attributes from herbarium, monographs, bibliography, as well as talking to experts, farmers, and consumers, but this has to be completed with breeding selection programs under field conditions to evaluate the relative potential of plant candidates. Later on, research on breeding for quantitative traits should be carried out in order to get rid of the limiting factors associated with the wild plant which are preventing to become a useful crop, as well as to strengthen the special and favorable traits of the future domesticated crop (DeHaan *et al.* 2016). For example, the hardseededness of the wild peas (*Pisum elatius*, *P. humile* and *P. fulvum*) from the Near East is a limiting factor, which produces low germination rates and poor crop establishment resulting in net yield loss (Abbo *et al.* 2011). By the contrary in tetraploid wheat (including the wild spe-

cies *Triticum turgidum* ssp. *Dicoccoides*), both non-brittle spike and free-threshing are favorable labor-saving traits that increase the efficiency of post-harvest processing (Tzarfati *et al.*, 2013).

Another example of the relative success in domestication of the perennial intermediate wheatgrass (*Triticum intermedium*) was described in the work of DeHaan *et al.* (2016).

## 15. GERmplasm BANK

As expressed above, in this situation of climatic change, germplasm banks play a primordial role to search and presumably to find valuable material with adaptation and plasticity to be fitted to the different conditions of future environments. The material preserved in genebanks may provide a source of important genes, which are waiting for their use in the genetic improvement programs addressed to obtain new crop varieties. The variability present in germplasm banks around the world is very large for most common crops and especially for major crops such as wheat, rice, corn, potatoes, grain legumes, horticultural crops, fruit crops, crucifer plants, grasses and forage species.

Current commercial varieties may fail as a result of the emergence of pests and widespread diseases, temperature and drought conditions, and reduction in the use of chemical fertilizers. The plant reserve existing in germplasm banks is supposed to have enough variability to find genes for resistance to these pests and diseases, tolerance to drought and greater efficiency in the use of nitrogen (Alonso Ferro *et al.*, 2007), as well as variability for forage digestibility (Brichette Mieg *et al.*, 2001).

As way of illustration, the Bank of germplasm (BG) of the Centro de Investigaciones Agrarias de Mabegondo (CIAM), A Coruña, Spain is described below. This BG can be an important reference to respond to the challenges of climatic change, since it has a great genetic variability that will be necessary for starting any program of genetic improvement of plants to be carried out in the North and Northwest of Spain. However the variability of the BG of CIAM is relatively small compared to the extremely wide genetic variability that can be found in the network of BG over the world.

In total, 4223 entries are conserved in the BG of CIAM, of which 693 correspond to maize; 669 to fruit trees of three different species, apples, pears, and cherries; 268 to four winter cereal species, wheat, rye, oats and barley; 293 belong to horticultural crops of six species, peas mainly, peppers, onions, tomatoes, cabbages and beans; and above all 2300 entries of several prairie species of grasses and legumes, especially the genera *Lolium*, *Festuca*, *Dactylis*, *Agrostis*, *Poa*, *Trifolium* and *Lotus*. The collection includes 700 entries of *Dactylis*.

## 16. CONCLUSIONS

There is today evidence of climatic change characterized by elevated CO<sub>2</sub> concentration and temperature, however there is uncertainty about the magnitude of the change concerning drought and rainfall periods over the different regions of the world. Plant breeding is proposed as tool for developing and adapting new varieties with complex traits adapted to future environments, such as higher temperatures and lower rainfall. Utilization of natural or induced polyploidy has demonstrated to be a good aid to increase vigor, yield, resistance to diseases, and adaptation of forage crops to stress. Development of synthetic varieties for forage plants seems to be a good option for achieving breeding goals in cross-pollinated crops. Hybridization between different species and within species is habitually used for increasing the vigor, the adaptation to environmental stress and the yield of cultivars. Hybrids between two species (e.g., from genus *Lolium*) produce usually a high rate of aneuploid plants in the F<sub>1</sub> generation. In successive generations, recurrent selection for raising the rate of balanced euploid plants in the F<sub>2</sub>, F<sub>3</sub>, F<sub>4</sub>... generations of the allopolyploid hybrids is needed for getting stable varieties. Commercial hybrid seed is preferred to synthetics provided that the additional yield and features compensate the extra cost of seed production. Exploration of new C<sub>4</sub> tropical species likely will help to produce forage in poor watering conditions. The use of <sup>13</sup>C isotopic discrimination seems to be a good indicator to breed for water use efficiency (WUE) in C<sub>3</sub> plants. Biotechnological tools, such as molecular markers, will facilitate to achieve breeding objectives sooner. Breeding suggestions for development of new varieties in C<sub>3</sub> and C<sub>4</sub> species adapted to future environments should consider the possible biotic and abiotic stresses generated by the climatic change. The interaction of increasing CO<sub>2</sub> with other environmental factors, such as water availability, temperature and mineral nutrition should be better studied for understanding the responsiveness of plants to elevated CO<sub>2</sub> atmosphere.

Domestication of wild species may be an alternative for developing a new crop to meet the requirements of environmental change. Crop domestication is a difficult task; however the use of rational strategies, including breeding for reducing the effect of limiting factors and strengthening favorable traits will help to achieve the desired objectives. Use of autochthonous germplasm as base material is crucial for providing genetic variability and genes adapted to environmental conditions.

## 17. ACKNOWLEDGEMENT

The authors thank to the Instituto Nacional de Investigación y Tecnologías Agrarias y Alimentarias (INIA) for the partial financing of the research projects RTA2012-00100-00-00 and RFP2014-00008-00-00, and the Complementary Action AC2015-00025-00-00.

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