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Summary

An overall analysis of the state of art of breeding and methodology in shrubs was carried out. First of all the review focuses on the breeding systems and it outlines the importance of improving knowledge on the latter for breeding shrubby species; moreover, a rapid, adoptable methodology is indicated for indirect determination of the breeding systems and the classification of various genotypes. Afterwards, the poor development of the breeding plans in shrubs is underlined; previsions of their improvement in relation to research trends are reported and the adoptable breeding plans are indicated on the basis of up to date knowledge and of the poor economical importance generally attributed to shrubby species.

The analysis continues on selection, which is unanimously considered as the prevalent expression of breeding activity in shrubs. On these aspects regarding forage shrubs, the review outlines the difficulties involved in terms of time, costs and efficiency; thus a synthesis of the methods used today for the estimation of biomass, which are suitable for selecting among different populations and within a single population, is reported; furthermore for each of these methods, the techniques and the level of accuracy and of suitability are indicated.

Criteria and methods used in selection for improving palatability are pointed out, outlining the importance of taking into consideration grazing animals for selection, the type approach for their management and the limits of the main selection methods used today.

Thereafter, basic criteria of selection for tolerance to salinity are analysed and the possibilities of using modern tecnologies for selection in controlled environments are indicated.

At last a critical, conclusive evaluation of the aspects analysed is done and the opportunity of developing new, known technologies for a faster improvement of shrubs is expressed.

1. Introduction

Many studies carried out in the last twenty years showed that shrubs may be considered as a modern agronomical tool for the exploitation and protection of natural resources of environments characterized by crop limiting factors.

The main problems involved in their utilization, however, are the following:

- How can the enormous potential of these wild-considered species be

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valorized?

- What kind of breeding improvements is it possible and convenient to accomplish, which methodologies should be used and what level of improvement may be expected?

The possibilities of giving an answer to these questions depend on the degree of complexity and on the level of understanding of the various aspects. On the basis of up-to-date knowledge, it is understood that the wide diversification of the reproductive mechanisms which regulate the gene flow and the modifications produced by rapid changes of the habitat are the reasons why shrubs are considered as a class of plants rapidly evolving towards polyploidy (Solbrig, 1972; Stutz *et al.*, 1979).

At present, the evolution to which the breeding systems and the polyploidic state are submitted, together with their wide diversification are the reasons why in the near future traditional screening methods are expected to be used (McArthur, 1988); however, the merging of traditional improvement criteria with principles of modern genetics of populations brought about the updating and the experimental approach of the techniques employed (Wyatt, 1983).

In this new framework, even if it is relatively easy to outline the fundamental phases of a breeding plan, there are difficulties regarding the choice and/or the definition of the methodologies adequate to the different circumstances which cannot be overlooked.

In fact already in the first phase of singling out and characterizing (evaluation) germplasm the difficulties are various and some regard:

- the definition of the reproductive system, insofar as it may range from completely selfing to obligate outcrossing, with flowers from bisexual to dioecious, with populations from monomorphic to trimorphic (Rosemary *et al.*, 1989);
- the understanding of the influence of biotic and abiotic factors on the gene flow, due to the need of examining the reproductive system of the plant in the most possible known ecological context;
- the determination of the methods of propagation (gamic and agamic), included micropropagation under study for some shrubby species;
- the definition of the relations of interdependance among the different plant characters both for characterization purposes as for selection in the different possible situations of breeding (productivity, quality, resistance

to biotic and abiotic stress, etc.).

In the subsequent phases the number and the degree of the difficulties increase in relation to the various aspects regarding the manipulation of the genetic material obtained, to the poor understanding of the mechanisms of genetical control of the characters at the different degrees of ploidy and to the methodologies of evaluation for screening purposes.

It is, in fact, known that the genetic variability of populations may be used to advantage through a simple phenotypical screening and a subsequent vegetative propagation, when the species makes it possible, or in the case of improved material with a high degree of homozygosis through controlled hybridization of known genotypes; in the latter case, both the use of this kind of approach as also the forecast of the hereditability of characters is difficult due to the small flower size, the several biological barriers and the variability in the polyploidic state of many shrubby species.

At last, as far as the methodologies to be used for screening are concerned, difficulties vary in relation to the objectives of the breeding plan, in many cases limiting the potential gain obtainable by breeding. The different characters bring about objective difficulties of evaluation due to intrinsic aspects and because their expression is markedly influenced by biotic and abiotic environmental factors and by the type of utilization; some examples are productivity, regrowth capacity and perennity in the case of forage shrubs. For some of these characters, generally some methodologies are adopted which are used in agronomical - forage type evaluations and the information obtained on the genetic material is not always realistic; moreover, the cost to selection efficiency ratio is rarely convenient.

Given the general need to identify evaluation techniques suitable for screening in different situations, easy to use, fast and chip, in the following pages those believed to be at present the most suitable will be examined. This investigation will include also a schematization of some improvement models and it intends to concentrate the attention on the complexity of the problems and represents a starting point towards a subsequent and more complete review and an occasion for a faster and more efficient development of shrubs.

2. Breeding system

The determination of the reproductive system represents the first step in any program of genetic improvement. As far as shrubs are concerned information is limited and only recently have these studies received a growing attention. The methods in use are mostly traditional ones and they regard the classification of the pollen vectors, the structure and characteristics of floral organs and the identification of pollination syndromes.

A statistical study for a rapid preliminary determination of the reproductive system was carried out by Pendleton *et al.* (1987) by singling out the variables useful for discriminating among genotypes having monoecious, dioecious, polygamous and perfect sexual systems. The study was based on a multivariate analysis model on 15 morphological and ecological characters of 351 species belonging to 45 different families; the results of the analysis are reported in the table 1 and they show how a preliminary indirect classification of different genotypes is possible.

Tab. 1 - Results from contingency table analyses for the association of sexual with each of the variables listed below.

	Woodiness	Height	Fruit (1) succulence	Geographic occurrence	Canopy layer
Dioecious	Shrub, large tree	Tall	No trend	Common	Overstory
Monoecious	Small and large trees	Tall	Dry	Dominant	Overstory
Poligamous	Subshrub	Short	No trend	Uncommon	Open
Perfect	Succulent, shrub	Short	Fleshy	Uncommon	Open, understory
P value	NA[2]	NA	0,0062	0,0000	0,0000

(1); result differed at genus level; from Pendleton et al., 1987.

3. Breeding plans

According to McArthur (1988), breeding plans for shrubs will continue to be based on traditional methods for same years yet, whereas modern biotechnologies may be used only later on. At present, in fact, studies on cellular and molecular biology carried out in U.S.A. on several species made it possible to put together revolutionary methodologies which may be used by biochemists and ecologists to better understand shrubs and the interaction shrub/environment, and by geneticists to study and increase existing germplasm (Carman, 1987). In parallel, studies on controlled hybridization with the use of chemical markers showed on the one hand the difficulties for the establishment of hybrids and, on the other, the achievable advantages (McArthur *et al.*, 1988).

However, the importance of natural hybridization in the process of differentiation of the species and the wide variability existing in many of them for phenological characters, plant growth, chemical composition, palatability, tolerance to repeated utilizations, anatomy and shape of leaves and inflorescences, ecc., make it possible to apply developmental programs for many shrubby species based on the collection and characterization of germplasm and on the selection and genetic manipulation of the gathered material.

Up to now the complexity involved in genetical manipulation of wild material in continuous evolution has limited the development of breeding plans for shrubs. However, the possibility of vegetatively propagating many shrubs and the recent ascertained studies on the micropropagation of some of them for selective purposes (Barrow, 1987; Wurtele, 1987), make it possible to apply the general lines of breeding plans used for cultivated species.

Simmonds (1989) advises for allogamous species, which may be propagated vegetatively, simplified models of mass selection through the same guidelines used in cultivated species with or without progeny tests for selection.

Panestos (1991) proposes a breeding plan for woody plants with sexual and asexual reproductive systems, pointing out the fundamental details of the program, the genetic implications and the advantages of vegetative propagation.

Other breeding plans are similar in the fundamental guidelines to those already mentioned and sometimes differ in relation to the degree of understanding of the species to be improved; one of them, for example, is in course of application (Stringi *et al.*, ongoing study) on *Medicago arborea* and it differs from the decribed ones insofar as it contains the same guidelines used for synthetic varieties.

4. Selection

Methodologies used for selection in the improvement of shrubs are various in relation to the species and to the characters under study; furthermore, they sometimes differ while using the same character.

In general selection efficiency is related to hereditability of the character and to the suitability of the selection method. It is well-known that productive characters are poorly hereditable and therefore selection methods are sometimes inadequate, especially at single plant level in obligate outcrossing, both for the low efficiency as for its limited convenience, also in terms of time and cost. Recent knowledge on the genetic structure of some shrubby species and on the use of new biotechnologies of cultivated crops determined the development of selection methods which improved stress tolerance to abiotic factors and biological value of production and reduce antinutritional factors content. In all cases, however, the choice of the method must take into account both the capacity of obtaining a selection gain, as also the economic significance of the selection efficiency achievable. As far as shrubs are concerned, these principles must be kept particularly in mind because of the difficulties of an objective evaluation of the characters, and because of time and cost demand. In fact, there are many problems which make the improvement of forage shrubs a difficult task, such as the variability existing in the populations, the quantity of biomass and its distribution on the branches and the rythm of formation during the productive season, the dimension and irregular vegetative structure, the susceptibility to abiotic stress and the low selection efficiency at single plant level.

In spite of the these and other limitations not mentioned, in various research stations other selection methods have been developed where validity, simple application, costs and time demands are frequently dependent on the complexity of the character to be improved. Among the characters of prevalent agronomic and forage interest, those believed to be the most significant are sythetically described and reported in table 2.

Tab.2 - Suitability of sampling tecniques for accuracy, vegetation type, selection and racommmended selection variables.

Assessment method	Accuracy	Vegetation type	Unit	Selection Variables	Suitability
Direct sampling	High	Efficient for homogeneous vegetation. Impraticable for large areas and high, sparse and variable vegetation.	Population, clones	Weight of samples.	Low-Moderate
Reference unit (C.E.V)	Moderate	Suitable for small-medium distinct shrubs. Not suitable for species rich areas.	Plant, population	Reference unit, biomass.	Moderate
Single plants (D.S.)	Moderate-high	Praticable in not dense vegetation with large discrete shrub or trees.	Plants	Branch girth basal stem diameter, crown and height.	Moderate
Visual estimation (D.S.)	Low-moderate	Usefull for small, obscure shrub vegetation.	Plants, population.	Biomass and structure, dimension and distribution.	Low-moderate
Image analysis	Moderate-high	Suitable for small-medium, not dense vegetation.	Plants, population.	Average diameter and height.	Moderate-high

(C.V.C) = Calibrated Visual Estimation; (D.S.) = Double Sampling.

4.1. Biomass production

Biomass production is one of the most difficult characters to improve through selection owing to the complexity of the vegetative structure, to height, to the presence of both woody and edible parts on the plant, to the interest for the different vegetational components, to the distribution of foliage biomass on the branches, to the size of the population sample, to time and means available, etc.

At present selection for the improvement of biomass production among populations of the same species or among samples of representative plants of populations which may be propagated agamically is based on techniques which are, in general, those used for evaluating the productivity of an area, the grazing potential, etc. For these purposes many different techniques are used and each one presents different degrees of advantages and drawbacks for every vegetational situation. In general, the utilization of these selection techniques made it possible to ascertain that the evaluation of biomass is inaccurate when sampling, however done, is carried out on ample and heterogeneous populations; moreover, structure, plant density, height and the complexity of vegetation increase the degree of difficulty and require the use of specific techniques; at last, the necessity of pointing out simple techniques to minimize time requirement for selection and to guarantee a correct evaluation of the biomass both of population samples as of single plants was acknowledged.

A recent review of the techniques used for agronomical evaluations of different vegetational formations was presented by Catchpole and Wheeler (1992); in this review the authors report on the advantages and drawbacks of these techniques, their relative cost and degree of accuracy, and they also give indications on their use for different vegetational types and sometimes criticize the adaptations proposed by various authors.

Not all of the techniques used for agronomical - biological type evaluations are adaptable to selection for biomass production improvement becuase many are the objectives of the evaluation, the degrees of accuracy required and the utilizations of the vegetational material evaluated. However, and in all cases, the techniques should have a high degree of accuracy when used for selection and be easy to use to reduce time-demand.

4.1.1. Direct sampling techniques

The direct sampling methods are up to now still considered the most suitable for an accurate estimation of biomass and an efficient selection of superior phenotypes of natural populations and of populations breeded as single-spaced plants, in spite of the fact that they present drawbacks such as their high cost and time requirement for cutting and determination of dry matter.

The advantages to selection offered by the accuracy of direct sampling decrease when the aplitutude of the vegetational formations, population variability, plant size and plant structure complexity and wideness increase.

As far as selection among populations of the same medium-low sized shrubby species is concerned, generally a high number of randomized destructive sampling is required for estimation accuracy; also in this case the size and the number of the samples increase with higher spacial variability of vegetation. As far as the size of the sample areas is concerned, Morris (1958) ascertained that estimation accuracy increased with smaller sized and more numerous sample areas, although the latter condition determines a higher work demand and border effect per sample area unit. In order to reduce the effects of variability on estimation accuracy Van Loon (1977) recommended to randomly delimit a series of quadrats separately for each area with a high, medium and low apparent biomass.

In populations with a thick and high vegetation a vertical profile may be used for direct estimation of biomass in sample areas using a three-dimensional cuboid frame (Catchpole, 1987) with movable sides; this method, however, is not suitable for plants with a complex and variable structure because of the weight of the branches pressed against the sides of the frame.

In these cases the method proposed by Sneeuwjagt in 1971 seems the most suitable because the cutters are attached to a pole in order to cut a cilindrical area of vegetation.

4.1.2. Calibrated visual estimation techniques

The calibrated visual estimation techniques are based on the calibration of reference samples to reduce the subjectivity of the estimator. They allow a rapid and satisfactory estimation at lower costs than the destructive sampling techniques. Due to their characteristics, they are generally suitable for selecting single shrubs among sparse populations belonging to medium-low sized species, whereas they are not good for selection in species-rich formations. Among the several methods used the Reference Unit Method is maybe the most suitable; it consists of a visual estimation of foliage biomass through the random selection of a sample of plants (meanly 40) which represents the range variability in height; the division of the sample in two groups and the utilization of one group for calibrating the relationship between the reference unit (a branch of a plant with 10-20 % of the mean biomass of the whole sample of plants - Andrew *et al.*, 1979, 1981) and the estimated unit equivalents of the foliage biomass of the plants; the utilization of the second group to validate the calibration relationships. This method is used by various researchers in different environments and shrubby species; it is considered suitable for the foliage biomass estimation of small, medium and discrete plants (Carpenter and West, 1987), as also for plants with a small, large-compacted and large-dispersed vegetative structure and, if the reference unit is the whole plant instead of a branch, it is good for estimating thick and compact foliage biomass, as well (Kirmse and Norton, 1985). According to Cabral and West (1985) the reference unit method is also suitable to estimate the grazable foliage biomass even in those cases of shrubs greatly varying in size and shape. The validity of the method was ascertained by Andrew *et al.* (1981) and by Kirmse and Norton (1985), by comparing it with other methods among which the double sampling with regression.

4.1.3. Double sampling techniques

Double sampling techniques are based on regression curves between biomass and easy to survey variables correlated with biomass. The parameters of the regression equation are obtained through destructive methods on a small sample of plants. A high number of measurements, on the other hand, is carried out for the easily measurable variables on an big sample of plants and the regression curve is used to estimate the mean biomass of an area. The method may also be used to estimate production components.

Generally new regression equations must be formulated for different vegetational units.

Some methods based on double sampling may be used to select between populations and between plants within a single population, through fast observations of easily measurable variables which are highly correlated to biomass production.

The nature of these variables highly correlated to biomass depends on the method used, according to Catchpole and Wheeler (1992).

As far as shrubs are concerned, some researchers demonstrated that these variables are height (H), diameter (W), vertical profile (HW) and volume (HW^2), (Seligman, 1986; Ungar and Genezi, 1990; Robledo *et al.*, 1991). These studies were carried out on several genera and species and they also showed that visual estimations of biomass and its components give the lowest predictory degree of the standing biomass (Seligman, 1986; Ungar and Genezi, 1990).

The estimation of the different components of biomass through easily measurable parameters was done by Robledo *et al.* (1991) on Anthyllis cytisoides in four different environments and the results were that basal area and maximum foliage zone volume were highly correlated to edible biomass whereas total volume was highly correlated to woody biomass.

4.1.4. Single plant estimation methods

These methods are based on the determination of the relations between biomass or its components (established through destructive sampling of representative plants) and easily measurable dimensional variables. In selection they are suitable for vegetatively propogated species planted with large layouts or for natural formations sufficiently sparse.

Some studies showed that the influence of these variables on biomass estimation varied in relation to the species and its shape and size. Considering trees and large shrubs with one or two main branches, the base diameter is the highest correlated variable (Brown *et al.*, 1982); in adult plants of small shrubs such as *Medicago arborea* it's the lenght of the branches and in young ones it's the total number of branches (Corleto *et al.*, 1990). The most suitable correlated variable for the biomass estimation of the branching system of a tree seems to be the circumference of the most representative branch (Attiwill, 1962). The base diameter seems to be suitable for trees but not practical for shrubs having a high number of stems branching at ground level. The measurements of the crown seems to be to most suitable for these shrubs, although these variables have a certain degree of subjectivity which may be controlled if two persons make the measurements horizontally and survey the irregularity of the shape of the crown. Each species may have a different regression equation, the form of which will depend on the shape of the shrub owing to the variability of the characters. In *Medicago arborea*, crown size, height, area and volume seem to be the variables mostly correlated with plant growth from one year to another and with seed production (Stringi *et al.*, 1991 and 1992).

4.1.5. Visual estimation methods

Visual estimation methods for biomass and its components have been studied by Bentley *et al.*, (1970) and by Seligman *et al.*, (1986); however, the low predictory of visual estimations pointed out by Seligman *et al.*, (1986), by Ungar (1988) and by Ungar and Genezi (1990), leads to the consideration that these estimations (proportional scores) are of poor significance for selection purposes. In order to surmount the limitations of subjectivity of the estimators, Fahnestock and Key (1971) tried to calibrate visual estimations; this technique in based on visual estimation of biomass and its components using scores given at photografic transparencies casted on a grill and then the regression of the scores with the measured biomass components; the method has the advantage of re-analysing the photografic transparencies and of being faster (one third) than destructive sampling.

4.1.6. Image analysis method

This method was defined by Cereti and Rossini (Rivista di Agronomia - in press) and maybe it represents the generation of fast, highly accurate techniques with a suitable cost to accuracy ratio. The method has some similarity with others mentioned insofar as the estimation of the standing and of the removable biomass with the analysis of the photograms requires the calibration of dimensional parameters easily measureable in the field (mean crown height and diameter) by means of the regression curve between dry matter of biomass and the variables obtained with the image analysis (mean area, mean volume, area of the base ellipse, volume of the ellipse-based solid). The accuracy degree of the estimations is very near to that of destructive sampling and the estimation time demand of medium duration. However, the method presents some limitations both for the great cost of the image analyser as for the obtainment of a high accuracy. In fact, in relation to light conditions, to plant rate, to the distribution of the edible biomass on the branches and to the complexity of plant structure and size, the analyser does not allow to estimate the portions of biomass obscured by the external ones. Moreover, this method requires the analysis of photogramms in laboratory and the determination of dry matter for the calibration of dimensional variables to be used in selection.

There is no doubt, however, that a new generation of low- cost analysers, easy to carry and capable of giving a direct estimation in the field through a simple reading of the machine would resolve most of the problems connected with biomass selection of species where breeding gives limited comebacks.

4.2. Palatability

According to Heady (1975) palatability is related to intrinsic plant characteristics (physical characters, chemical components, season of growth) and is influenced by availability and abundance, whereas selectivity is in relation to the reactivity and characteristics of the animal (experience, age, species, state of health, condition). Numerous studies carried out on the relations between chemical composition and palatability are not always unanimous; however, the positive correlation between proteic content and high palatability is very frequent. Among the different species there is a considerable variability in the factors which contribute to palatability (Voight, 1975). To improve this character the fundamental guidelines of a breeding plan aimed at valorizing the existing variability may be the following:

- gathering of information from breeders on the preference of the different animals and collection of the mostly preferred germplasm;

- selection in the field using animals and evaluating the degree of utilization;
- selection in laboratory among phenotypes previously selected in the field on the basis of the antinutritional contents (essential oils, saponins, phenolics, phenolic resins, sesquiterpenes);
- reproduction of the selected, highly palatable genotypes also by means of new techniques of micro-propagation (Wurtele *et al.*, 1987).

It is understood, however, that this kind of approach involves difficulties deriving from factors related to selectivity, from technical problems, from the animal management cost and from unknown factors which determine differences in palatability.

Methodologies used for field selection are various; some are based on the estimation of the degree of grazing intensity after a long period (2 years or more) of utilization (Otsyina, 1983); others also on the priority and the duration of grazing of the animal (Forti, 1971).

In all cases the following requirements must be considered:

- establishing in the estimation areas conditions of approachability for the animals so as to not impair preference;
- picking always the same animals and an adequate livestock number/ha;
- repeating observations for different years and a long time (2 productive seasons or more);
- adopting random distribution with replications of the plants in cases of selection between different species or clones.

The estimation of grazing intensity is based on the same techniques already described for that of biomass. In reference to the double sampling techniques, for example, Forti (1987) evaluated the palatability of different species by means of estimating the biomass grazed by sheep and goats, with observations before and after grazing of plant parameters, the construction of the regression curve and the conversion of the visually estimated data of the plants in the field according to the technique decribed by Seligman (1986).

The analasys of the results of this and other similar studies in Israel pointed out a further reduction of the biomass predictory degree of visual estimation after grazing; in fact, it was observed that herbivorous animals utilize plants irregularly and that grazing regularity depends on:

- livestock number/unit area;
- utilization intensity;
- palatability of the various parts (fruits are less palatable than leaves and more than twigs);
- the approachability and the grazing habits of the animals (generally growing shoots are grazed and only on one side, whereas the remaining part of the plant is untouched, Seligman *et al.*, 1986).

Other techniques similar to the reference unit method which are used to estimate the biomass grazed by herbivora could be adopted for selection between populations with a different palatability degree.

Generally techniques allow to estimate the utilization percentage (U), by means of adopting a high livestock number and continuous or repeated grazing for a duration equal to that of the productive season; random selecting a sample of plants (50/ha); picking on each plant a sample of a reference branch of the edible material. The estimation of the percentage of grazed biomass is calculated in different ways on the basis of the observations surveyed on the reference branch. According to Jensen and Urness (1981), the grazed percentage may also be estimated through measurements on a limited, but replicated, number of plants, of the base diameter (Db), diameter of the twig tip (Dt), diameter of the twig tip after grazing (Dp), mean lenght of the twigs before (Lf) and after (Ls) grazing. On the basis of the observations, U = 100(Dp-Dt)/(Db-Dt), or U = 100(Lf-Ls)/Lf. The regression analysis of the observed variables with the dry matter of the biomass of the twigs before and after grazing showed that the diameter at the grazed tip is highly correlated to the biomass removed but not to the biomass which remains.

On the other hand, Provenza *et al.*, (1991) calculated the percentage (U) by means of picking one branch/plant with meanly 100 cm of edible biomass, measuring its lenght before and after grazing and using the formula U = (1-(A/B))100, where B is the mean lenght before grazing and A is the mean lenght after grazing; the same author tried to put together an alternative and cheaper method by means of calculating on 40 branches the relations existing between diameter, lenght and weight (both air and oven dried) of grazed twigs.

In relation to grazing priority and duration as palatability discriminating variables, Forti (1971) adopted a technique to select among different species, based on the use for each species of a sufficiently wide area repeated a high number of times and on the estimation of palatability during 12 months according to the chronological order of utilization and intensity of grazing.

Whatever the technique to be used to select in the field, to correctly approach palatability improvement, it is indispensable to test the selected material in laboratory for a second cycle of selection based on the contents of antinutritional factors.

As far as *Medicago arborea* and *Atriplex spp.* are concerned laboratory selection for saponins may be done using an effective and rapid method proposed by Jurzysta (1979) and simplified by Gorski and Jurzysta (1988), which is at present used on *Medicago arborea.* Owing to its simplicity and rapidity the method allows to test a high number of samples/day and is based on the use of a leaf portion of 20 to 100 mg of each plant to be tested. The influence of the species, of the type of saponins and of other aspects must be considered in order to extend this method to shrubby species.

4.3. Abiotic and biotic factors

The presence of saline soils characterizes wide areas of arid and semi-arid environments, meanly 53 million hectares worldwide of irrigates areas and territories with changing hydrological conditions.

The capacity of many shrubs to tolerate high saline concentrations, the new possibilities of production utilization (O'Leary *et al.*, 1988) and the new awareness on environmental safeguard contribute to the developement of studies on improving tolerance to salinity of these species. However, shrubs may be generally considered a vergin field for this aspect and an enormous variability exists to be exploited; there is for this and for other characters the possibility of having limited comebacks in change of enormous efforts.

Nevertheless, in U.S.A. (Plummer, 1966) and in Western Australia (Malcolm *et al.*, 1984), breeding plans have been carried out that allowed to single out species tolerant to salinity and drought.

A rapid view of the available scientific knowledge shows that the most important phases of a selction program for salinity resistance are the following:

- a collection of seed samples representing all likely useful plants;
- test sites that truly represent the problem areas;
- adequate facilities and methods for test work;
- meaningful selection criteria.

An aspect unanimously believed fundamental is the choice of the test sites which must be representative of the problems of the area related to soil type, climate and degree of salinity in order to reduce the effects of variability of the soil and climate which are high in saline soils and in arid and semi-arid environments. For these reasons Judd and Judd (1976) recommend a high number of replications (20) and a long observation period (2 to 30 years).

Selection criteria may be based on the estimation of the following characters: survival, growth, tolerance to cold, flowering and seed production, natural reseeding, grazing value, ion composition, grazing recovery, growth habit, digestibility, resistence to pests or diseases, salt and waterlogging tollerance, drought tollerance, resistance to sand blasting and palatability (Malcolm, 1989).

Breeding plans for irrigation with saline water based on traditional criteria related with tolerance to salinity and waterlogging and with other characters such as vegetative growth, seed and biomass production have been carried out in U.S.A. by O'Leary (1966), Watson et al. (1987), Watson (1990) and in Israel by Forti (1986); whereas selection on several species regarding the use of sea-water for irrigation was done by O'Leary *et al.*, (1985).

The possibility of exploiting both the ample variability of shrubs in tolerance to salinity and the immense resources of saline soils and water through selection was ascertained in all of these studies together with the need and difficulty of predicting over a long period of time the effects of continued irrigation with saline water on the soil, on productivity and producion quality.

In interaction with traditional criteria, new breeding methods have been developed and applied; among them the method of Newton and Goodin (1986) gives all of the details of the techniques used: the first step is to select for adaptability (survival, vigor and production) among 28 different populations of *Atriplex* in four different environments, secondly the genotypes which survive are tested in laboratory and then single cells and axil leaves are micropropagated in order to select in controlled conditions both for resistance to drought and salinity.

The results of this study on *Atriplex* populations showed that in vitro culture of single cells and of tissues may be considered a valid instrument for drought and salinity resistance selection.

Knowledge is very poor in relation to biotic factors mostly because shrubs are not yet seriously considered as cultivated species.

However, many species present disease and pest problems; in relation to *Atriplex*, for example, there are insects which damage shoots and roots, together with locusts, coccids and rodents; the latter consume in the mediterranean area up to 2500 kg/ha/year of fresh matter (Le Houerou, quoted by O'Leary *et al.*, 1988).

As far as *Medicago arborea* populations of the mediterranean area are concerned, insects eating twig piths and shoots were observed and considerable fungus infections, which go up through the roots and into the trachea system, determined graudual deterioration, clorosis and death in occurrence of waterlogging in clay soils.

These aspects have not yet been studied for breeding purposes but it is time to do so, adopting in the first phases breeding techniques used for cultivated species when improving biotic stress resistance.

5. Conclusions

The considerable influence of the sudden changes of habitat on the evolution of the breeding systems, on the gene flow and on the appearance of new individuals with higher levels of ploidy are basically responsible for the continuous, enormous release of genetical variability of many shrubby species.

These factors, however, on the one hand allow great potential possibilities of developement considering selection alone, but on the other, together with the poor knowledge in the field of genetics, represent the fundamental factors which limit the exploitation of the available variability.

These aspects and the present modest economical importance of shrubs do not allow, in fact, the application of modern technologies, but only of simplified breeding plans in the short and medium term. In this sense, the activity carried out in research stations showed that the efficiency of selection depends on the character to be improved and on its genetic control, on the breeding system, on the life cycle of the species and on the breeding method used. In fact the analysis of the methods used in selection, in spite of the promising prospectives of utilization both of modern technologies (micropropagation) in selection for resistance to abiotic stress (salinity, temperature) in controlled environments and of rapid chemical laboratory analyses for palatability selection, conferm the existance of several limitations in biomass selection.

In relation to biomass estimation, the advantages of a high accuracy for improving results in selection offered by destructive sampling of single plants are limited by the high time demands and costs required and are overmore reduced as variability, size and plant structure increase.

Non destructive methods are generally faster and cheaper that destructive ones, but they achieve moderate accuracy degrees and, probably, low contributions to selection. Moreover, because of their intrinsic characteristics, some of them could be suitable for selecting single phenotypes in sparse populations of medium-low shrubs (reference unit), others for the selection of single plants or groups of plants also on the basis of biomass components (Double sampling techniques).

The Image analysis method is potentially interesting because of the necessity of having fast and cheap estimation methods with a high accuracy degree and of the possibility of re-analysing the photos.

A new generation of cheap analysers, easy to carry and capable of memorizing and analysing the estimations directly in the field will probably allow a considerable advance in shrub selection.

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