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Agronomic, qualitative (β-ODAP) and molecular variability in grasspea populations of the Marche region (central Italy)

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ABSTRACT

Grasspea is an interesting crop for rational crop rotations in inner areas of the Marche region. However, breeding efforts are needed to improve its agronomic performance and to lower as much as possible the neuro-active amino acid β-ODAP in the seeds. Since the year 2000, a germplasm collection and evaluation program has been carried out. Accessions were evaluated by field trials for morphological, physiological and agronomic traits and for seed β-ODAP content. Significant variability was detected. In particular, populations collected in local farms showed a higher seed production compared with unadapted accessions. β-ODAP content varied between 0.26% and 0.53%. Moreover, AFLP molecular markers were applied to investigate genetic variation characterizing the populations included in the collection. In the year 2008 a breeding plan was started using two household populations, one with the lowest average β-ODAP content and one with the highest agronomic performance. Based on these results, a within population analysis for this trait will be started using the accession with the lowest β-ODAP content. Interesting information was also gathered using AFLP molecular markers, since a wide range of genetic variation supported the possibility of starting a mapping project aimed at the identification of genes of the biochemical pathway for β-ODAP.

1. Introduction

Grasspea (*Lathyrus sativus* L.) is a traditional legume crop which was quite common in central and southern Italy until the middle of the 20th century (Bozzini, 1997). The grain was used for human consumption and the hay for animal feeding. Recently this crop has been almost completely abandoned due to the expansion of other grain legumes such as field bean (*Phaseolus vulgaris* L.), pea (*Pisum sativum* L.) and chickpea (*Cicer arietinum* L.). Availability of improved varieties, abandoning of marginal lands followed by the expansion of high input agricultural systems and the risk of lathyrism (Cantani, 1873) could be considered as the main causes for the reduction of grasspea cultivation.

However, traditional cultivation of grasspea is currently confirmed by the presence of local populations still cultivated in small farms for household consumption (Granati et al., 2003; Polignano et al., 2005; Tavoletti et al., 2005). However, grasspea has interesting agronomic peculiarities that make it a crop with promising potential as food or feed in inner areas of central and southern Italy (Hanbury et al., 2000). In particular, its high level of drought tolerance, adaptation to poor soils and high level of protein content suggest that grasspea could be reintroduced in balanced crop rotations with cereals in inner areas where irrigation is not feasible. However, in order to be competitive with alternative crops, grasspea should be improved for important agronomic traits such as seed yield and ability to compete with weeds, but the highest effort should be directed to decrease or eliminate the neurotoxin β-ODAP (3-*N*-oxalyl-L-2,3-diamino-propionic acid) which is considered the cause of neurolathyrism (Yan et al., 2006).

For these reasons research has started in the year 2000 to collect local grasspea germplasm still cultivated in small farms of the Marche region (central Italy) for household consumption. Field evaluation of collected accessions was carried out in different environments of the Marche region to gain information about the agronomic potential of grasspea under sustainable or organic agricultural systems. Moreover, screening for the presence of β-ODAP in seeds of different accessions was carried out in collaboration with ENEA C.R. Casaccia – Rome to evaluate the presence of potential genetic variability for this important trait to be explored in further breeding programs. Genetic variability among and within accessions was also tested by the use of AFLP (Amplified Fragment Length Polymorphism) markers (Vos et al., 1995) to quantify the genetic relationships among accessions.

The potential of grasspea as protein concentrate in animal feeding has been tested by one feeding trial carried out with beef cattle

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of Marchigiana breed raised in an organic farm of the Marche region. Information on the effectiveness of grasspea grain as animal feed is important to address breeding programs since the characteristics of improved lines could differ between the two final potential uses: human consumption or animal feed.

Molecular, agronomic and quality (β-ODAP) information was comprehensively used to start a breeding program in the year 2009 aimed at the selection of superior lines which could be used as starting point for the expansion of grasspea cultivation in the Marche region. This effort follows the inclusion since 2008 by the Italian Ministry of Agriculture and Forestry of grasspea in the list of Traditional Agricultural Products of the Marche region, which could represent a significant incentive for the cultivation of grasspea as local and typical product.

In the present paper a summary of the results obtained up to now, together with possible future evolution of the research on grasspea in the Marche region will be presented.

2. Materials and methods

2.1. Germplasm collection and first field trial

Collection of grasspea germplasm available in the Marche region started in 1998. Collected populations were subdivided in two main groups, household (H) populations collected in small farms cultivating them for family consumption, and commercial (C) populations collected in market-oriented farms or in local stores. One population, here coded C3, collected at Serra de' Conti (province of Ancona) was cultivated from market-oriented farms organized in a Cooperative. This population was characterized by small seeds with a very thin tegument which facilitates cooking and it is used in a local celebration entitled "Festa della Cicerchia" (Grasspea Feast) held every year in November at Serra de' Conti (Province of Ancona). A list of accessions collected during the 1998–1999 period is reported in Tavoletti and Capitani (2000). Subsequently other accessions were added to the collection which is stored in the Plant Genetics laboratory of the SAIFET Department – Marche Polytechnic University.

Morpho-physiologic and agronomic evaluation of the germplasm collection started in 1999 using a limited number of accessions available at that time, 4 household and 4 commercial populations (Tavoletti and Capitani, 2000). The trial was carried out at Serra de' Conti using both solid seeded (20 plants/m^2) plots and two rows plots with spaced plants (1 m distance between and within rows). Solid seeded plots were used to evaluate flowering time, seed production and 100 seed weight, whereas the following morphological and production traits were evaluated at the level of single plants on spaced plant plots: flower color, seed color, flower peduncle length, leaflet length and width, leaflet length/width ratio, longest stem length, first pod height, number of stems per plant, number of pods per plant, pod length, number of seeds/pod, seed production per plant. Data were analyzed by Analysis of Variance and results concerning 100 seed weight and seed production will be discussed. A comprehensive discussion of the overall results of this first trial including also Principal Component Analysis based on Pearson Correlation Coefficient matrix is reported in Tavoletti and Capitani (2000).

2.2. Second field trial

Based on information gathered from the first field trial, in the year 2000 field evaluation of 16 populations, 7 household populations and 9 commercial ones, was carried out at two locations of the Marche region, Polverina and Serra de' Conti (Tavoletti et al., 2005). Nine traits were evaluated in solid seeded plots: number of stems per meter (NSM), longest stem height (LSH), leaflet length and width (LL and LW), first pod height (FPH), pod length (PL), number of seeds per pod (NSP), seed production (SP) and 100 seed weight (100SW). Moreover the following traits were evaluated in single plots with spaced plants: flower color (FC%: percentage of plants with purple flowers), flower peduncle length (FPL) and flowering time (FT). Attention was addressed to the comparison of the two groups (household vs. commercial populations) to confirm the clear differentiation suggested by the first field trial. Data from solid seeded plots were analyzed by ANOVA (Tavoletti et al., 2005) whereas population means of all 12 traits evaluated were used to carry out a Cluster Analysis (Euclidean Distance matrix and UPGMA clustering method) and a Principal Component Analysis, based on the Pearson Correlation Matrix.

2.3. Seed β-ODAP and protein content

Information on neurotoxin β-ODAP content was necessary to complete our survey on the grasspea collection. In collaboration with ENEA C.R. Casaccia – Rome, a colorimetric method (Granati et al., 2003) was applied to quantify seed β-ODAP content of 16 populations (Tavoletti et al., 2005). Moreover, seed protein content of each population was determined as total Kjeldahl nitrogen percentage ($N \times 6.25$; dry matter%). Correlation of β-ODAP and protein content with seed production at each location was evaluated (Pearson Correlation Matrix).

2.4. Feeding trial on beef cattle

To test the possibility of including grasspea in the group of grain legumes potentially useful for animal feeding systems based on locally produced raw materials, a feeding trial was conducted in the year 2004 in an organic animal farm of the Marche region (Province of Pesaro-Urbino). The feeding trial was carried out comparing three rations (Table 3) including species which could be cultivated in inner areas of the Marche region characterized by absence of irrigation: grasspea, field pea and faba bean as protein concentrate and barley as energy concentrate. Oat hay was used as forage and furnished "*ad libitum*" to the animals. Three homogeneous groups of five calves each (3 of Marchigiana Breed and 2 half-breeds) were used to evaluate average growth rate which could be realized using rations based on locally produced raw materials.

2.5. Molecular marker analysis

To verify if the differentiation between household populations and commercial ones could be detected also by molecular approach based on DNA analysis carried out with DNA markers, 20 populations (17 collected in the Marche region and 3 in the Abruzzo region) were analyzed by AFLP markers (Vos et al., 1995). For details on the method of molecular marker production and analysis refer to Tavoletti and Iommarini (2007). Polymorphic markers were used to carry out Cluster analysis, based on the Dice Coefficient similarity matrix and UPGMA (Unweighted Pair Group Method using Arithmetic Averages) clustering method. Moreover a matrix of polymorphic marker frequency was generated and used to perform a Principal Component Analysis (PCA), based on the Pearson Correlation matrix. In the present paper, results of PCA concerning the subset of 16 populations also evaluated for β-ODAP content are reported. This permitted a comparison of molecular results with multivariate analysis concerning quantitative morphologic, physiologic and agronomic traits. Since DNA analysis involved a large group of 109 AFLP markers, the correlation matrix among the markers will not be included, and results will be summarized by a bi-dimensional graphical representation where clusters grouping commercial and household populations will be evidenced.

3. Results

3.1. First field trial

Seed size and seed production are commercially important traits. Populations with small seed size are preferred for human consumption, especially in organic markets, whereas large seeded populations are mainly used as feedstuff because seed integument is thicker than in small seeded ones. A significant among populations variability was found for both these traits: 100 seed weight ranged from 22.85 to 39.45 g and seed production ranged from 2.257 to 5.434 t/ha (Table 1). Correlation between these traits was low ($r = 0.22$, not significant) though the highest producing population (FER1) showed also large seeds, and population with the smallest seeds (SDC4) was characterized by a very low seed production (Table 1). Moreover, multivariate analysis showed that household populations formed a cluster clearly separated from the

Table 1
Results of field trial 1 concerning important agronomic traits: 100 seed weight (100SW) and seed production (SP). (Modified from Tavoletti and Capitani (2000)).

Location of collection	Accession Code	Category*	100SW** (g)	SP** (t/ha)
Arcevia	ARC1	H	39.45 a	2.864 de
Fermo	FER1	H	32.65 b	5.434 a
Serra de' Conti	SDC2	H	29.71 c	4.548 bc
Serra de' Conti	SDC1	H	26.66 d	5.220 ab
Colfiorito	COL1	C	24.61 de	2.257 e
Penne	PEN1	C	23.40 e	3.025 d
Serra de' Conti	SDC3	C	23.35 e	3.937 c
Serra de' Conti	SDC4	C	22.85 e	2.484 de

* Household (H) or commercial (C).
** For each trait, means with at least one letter in common do not differ statistically (LSD, $P < 0.05$).