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Yield and seed chemical composition of *Lupinus mutabilis* in Portugal

Produção e composição química de sementes de *Lupinus mutabilis* em Portugal

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ABSTRACT

Lupinus mutabilis Sweet (tarwi), being cultivated for thousands of years in the Andean region, is regarded as a potential crop for Europe, especially because of its high protein and oil content. The objective of this study was to revise the productivity and seed composition analyses of several tarwi accessions conducted over several years, in a Mediterranean environment (Lisbon, Portugal), in order to select more suitable lines. The productivity, although low (bellow 2 t/ha, in average), and variable (ranging from 0.26 to 6.0 t/ha), indicates that it should be possible to obtain lines with productivity levels high enough to make of this species a non-irrigated autumn-sown legume crop. These results are more encouraging when compared with the lower yields obtained in central Europe, where indeterminate growth also often causes unsurpassed difficulties. The chemical composition of tarwi seeds obtained in this Mediterranean environment reaches very high levels both in the protein content (up to 50%) and in oil (up to 19%). It is possible to select plants for higher oil levels with little reduction on the protein content. Seeds seem to have a slightly lower protein content than when obtained under central European conditions, but with oil contents consistently higher. Presently, the main obstacles to be transposed in order to make tarwi a crop for the Mediterranean area are: to increase cold and frost tolerance, in order to adapt it to colder winters than those in coastal areas; to increase seed yield and oil content (without reducing protein); to increase resistance to pests and diseases and a better competition against weeds; and to optimise the plant architecture in order to maximise pod and seed set without compromising productive flexibility (so much necessary to face the typical Mediterranean climate interanual variability).

Key-words: Andean lupin, tarwi, protein and oil; crop adaptability.

RESUMO

A espécie *Lupinus mutabilis* Sweet (tarwi), cultivada há milhares de anos na região Andina, é considerada uma cultura com potencial para a Europa, especialmente devido ao elevado teor proteico e oleico das suas sementes. Com este trabalho pretende-se rever os estudos realizados durante vários anos com vista à caraterização da produtividade e composição química das sementes de diversos acessos de tarwi, cultivados em ambiente Mediterrânico (Lisboa, Portugal), com o objetivo de selecionar linhas mais adequadas. A produtividade, apesar de baixa (menos que 2 t/ha, em média), e variável (0,26 a 6,0 t/ha), demonstrou que é possível obter linhas com produtividade suficiente para tornar esta uma cultura de outono-inverno de sequeiro. Estes resultados são mais encorajantes que outros obtidos na Europa central, onde o hábito de crescimento indeterminado origina com frequência dificuldades inultrapassáveis. A composição química das sementes de tarwi obtidas nestas condições Mediterrânicas alcança elevados teores proteicos (até 50%) e oleícos (até 19%). Os resultados obtidos indicam a possibilidade de selecionar plantas com maiores níveis de gordura nas sementes sem redução do teor proteico. Quando comparadas com semente produzidas no centro da Europa, as sementes produzidas em Portugal apresentam teores proteicos ligeiramente inferiores mas teores oleicos consistentemente superiores. Os principais desafios que se colocam a tarwi para a sua adoção enquanto cultura para o clima mediterrânico são: o aumento da tolerância aos frio e à geada, permitindo a sua cultura em zonas de maior continentalidade; o aumento da produtividade e do teor de gordura (sem comprometer o teor proteico); o aumento da resistência a pragas e doenças e uma maior competitividade com infestantes; e a otimização da arquitetura da planta, maximizando o vingamento de vagens e sementes, sem comprometer a flexibilidade produtiva (tão necessária pra fazer face à variabilidade climática interanual, típica do clima mediterrânico).

Palavras-chave: tremoceiro-dos-Andes, tarwi, oleoproteaginosa; adaptabilidade cultural.

INTRODUCTION

In an European context of strong dependence on imported plant protein products, a search for protein rich crops led to look at a long time cultivated plant (for more than 3000 years, in the Andean region), tarwi (*Lupinus mutabilis* Sweet), whose cultural and seed chemical composition qualities had attracted the attention of European lupin researchers and breeders. These qualities are specially a high seed content concerning protein (up to 50%) and oil (up to 25%) (Bellido, 1986; Santos *et al*., 1997).

Within lupins, tarwi stands out for being almost the only American cultivated species, among 300 wild ones (Dunn, 1984). As other lupins, it can stand water stress conditions and low fertility soils, allowing its use in areas where efforts are made to have an each time more sustainable agriculture (Gross, 1982). Nevertheless, tarwi poses some breeding challenges, namely due to: its high seed alkaloid content; its low and variable productivity, due to an indeterminate growth; and its low resistance to frost.

The scope of this work is to present this species, covering its potentialities and problems as a potential future crop for Mediterranean region (Portugal), with a special concern in seed quality and productivity.

MAIN CHARACTERISTICS

Morphological types

Tarwi is characterised by an important genetic variability within the Andean region, with different morphological types found (Gross, 1982):

- Small plants (less than 60cm high, no branches and short growing period), specially in the region of Potosí (Bolivia) and other areas over 3500m with low temperature and rainfall;

- Branched and tall plants (but producing more than 50% of the seed weight in the main stem), with a medium growing period, appearing in the Andean valleys of Bolivia and Southern Peru and;

- Highly branched plants, more than 1.8m high, long growing period, small part of seed production coming from main stem, narrower leaflets than in the other groups, nearly round and less coloured seeds, higher resistance to anthracnose and rust, being found in Colombia, Ecuador and Northern Peru, in warmer and frost free climates.

Flower colour is variable and varies also along flower maturation, hence the epithet “mutabilis” (Figure 1).

Growth habits

Tarwi plants can grow and produce branches and flowers continuously (whenever environmental conditions allow it), or, in the other hand, they may stop growing at a certain moment (even under

Figure 1 - *Lupinus mutabilis* plants at first flowering, depicting colour variation along flower maturity.

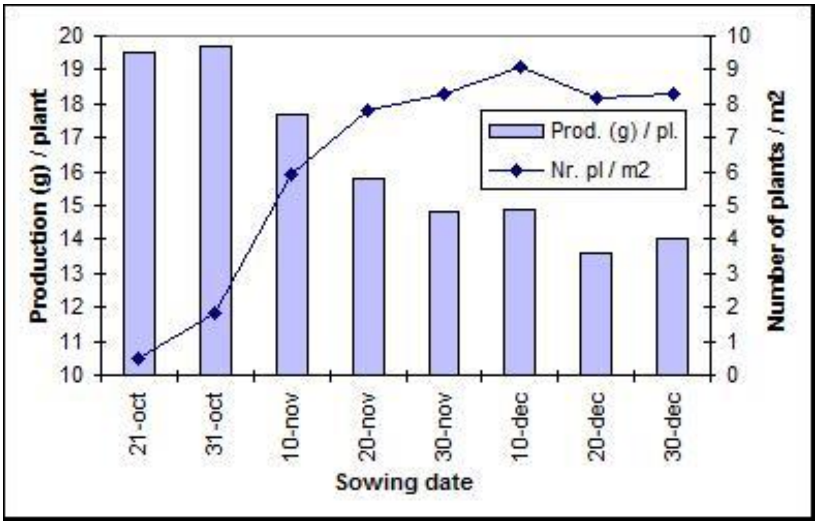

Figure 2 - Comparison between production per *Lupinus mutabilis* plant and plant density obtained for eight sowing dates (average of 11 cultivars).

favourable environmental conditions). These are indeterminate and determinate growth plants, respectively (Huyghe *et al*., 1994). The architecture of tarwi plants was studied under Portuguese Mediterranean conditions, showing important genetic variability among the analysed lines, ranging from small plants with short branches to high, very branched plants (Vaz Patto *et al*., 1999).

The advantages of those two kinds of structure (determinate and indeterminate) have been largely discussed for the different lupin species, the same applying to tarwi. This way, indeterminate plants, having simultaneously growing branches and filling pods and seeds, show a smaller efficiency in photoassimilate partitioning (Gross, 1982). However, this distribution of seed production by the different branch level (and so, during a longer part of the growing season) allows these indeterminate plants to be better suited to face inter-annual meteorological variations. Because of this, determinate plants are more adapted to Northern and central European conditions (also because they allow maturity to occur), while indeterminate plants suit better Southern (Mediterranean) Europe (where summer water stress makes unnecessary a genetically determined maturation). This is quite evident in the results of variety comparison trials that were carried out simultaneously in Portugal, France, United Kingdom, Germany and Poland n three years, where the highest yield in Portugal was obtained in a branchy line that was the poorest in the Northern European countries. On the other hand, the smallest and less branched line, one of the less productive ones in Portugal, reached the highest yield in Poland (Martins and Silva, 1994; Hardy *et al*., 1998; Talhinhas *et al.*, 1999).

Growth cycle

As other lupin crops, tarwi plants have a long growth cycle, which make early sowing advisable. According to its characteristics, this species must be faced as an non-irrigated Autumn-sown crop for Mediterranean climate areas, being October and November the recommended sowing months. Our results (Talhinhas *et al*., 1999) show that latter sowing dates cause smaller production per plant (since lesser time is available for branch production and pod filling), but earlier sowings originate high plant mortality. The combination of these two factors leads to the conclusion that an intermediate sowing date (such as November, under Mediterranean climate) will attain higher productivity (Figure 2).

The higher mortality of earlier sown plants may be due to the fact that these plants, sown still under mild Autumn temperatures, do not undergo an acclimatisation process to cooler Winter days when they are still at the plantlet stage, dying latter on Winter colder days. Under this hypothesis, plants arising from later sowing dates (such as December ones) would already have germinated under such conditions.

Environmental needs

In the Andean region, tarwi is sown in the warm and rainy season and harvested in the dry season, which is also characterised by a large daily thermic amplitude (i.e., cold nights). Under Mediterranean climate conditions, Autumn-sown plants endure low temperatures on their earlier phases, which are opposed to their original environmental conditions. This is why these plants show little tolerance to the Mediterranean winter frosts and colder days. In Portugal, tarwi can only be cultivated successfully in nearly frost free regions. There was little success when these plants were grow in further continental (and so, colder) areas of the Iberian Peninsula [Córdoba, Spain (Bellido, 1992) and Beja and Bragança, Portugal (unpublished data)]. Nevertheless, the very high and cold Andean areas where these plants can be found suggest that it may be possible to find plants with stronger levels of frost tolerance. Laboratory tests simulating cold nights (-5ºC) show the existence of genetic variability concerning frost tolerance, revealing lines with more than 70% of plants surviving to such conditions, within segregating lines obtained by induced mutagenesis (Santos *et al*., 1999). This way, it seems possible to obtain plants with higher frost tolerance, enlarging the potential areas for this crop to further northern and less coastal areas.

Mediterranean temperature conditions also affects negatively these plants at the maturation stage, since the lack of cold nights (under 9.5ºC) in spring/summer reduces the accumulation of oil in the seeds. It is also known, at least in laboratory conditions, that strong water stress at maturity can reduce seeds oil in about 50%, when compared with well watered plants (Carvalho *et al*., 1997; Borek *et al*., 2015).

CHEMICAL COMPOSITION

Tarwi seeds present higher (or at least, similar) protein and oil content values than other lupins and even other legumes (like soybean, common bean, pea or faba bean; Borek *et al*., 2015; Carvajal-Larenas *et al*., 2016).

As for most legumes, tarwi seed proteins present low amount of sulphured amino acids (methionine and cysteine) when considering human or animal nutrition. Nevertheless, its biological value is higher that the one recorded from *L. albus*, *L. angustifolius* and *L. cosentinii*, and comparable to soybean (Savage *et al*., 1984; Santos *et al*., 1997) and the digestibility is quite high (93.4%) for a legume (Gross, 1982). Tarwi protein is mainly composed by globulins and albumins, representing respectively 43-45% and 8-9% of total seed nitrogen (the remaining is present in non proteic nitrogen components, such as alkaloids) (Santos *et al*., 1997).

Within lupins, tarwi is the only species capable to reach the value of 18% oil content (the minimum for the industrial extraction). The comparison between tarwi seeds oil quality and other plants shows that tarwi oil: does not need an industrial removal of the linolenic acid, present in soybean, rapeseed and *L. albus* and which is harmful for oil industrial stability; is more equilibrated than soybean and rapeseed specially in what concerns oleic acid; and does not have any erucic acid (toxic) as in *L. albus*. Even when compared with other more currently used oils in human nutrition, tarwi presents a higher quality (as compared to cotton, sunflower or maize) or similar (peanut and oak seeds), being only inferior to olive oil (Talhinhas, 1994).

A total of 149 Peruvian tarwi germplasm accessions were cultivated in Lisbon (38°42′32.5″N 9°10′58.5″W, 60 m) in 1989 and 1990 (Silva, 1991), studied for morphologic and agronomic traits, and their seeds analysed for oil (Soxhtec method, 45min. extraction) and protein (Kjeltec method). Oil appears to be negatively correlated with yield components. Taller and bigger plants tend to be richer in protein, while smaller plants are richer in oil (Martins *et al*. 1992). Oil and protein are not correlated (Figure 3) (Martins *et al*. 1992; Neves-Martins *et al*., 1997), allowing to select for higher oil amount with the possibility of preventing any major loss in the protein content, both because the regression coefficient is not high and the correlation varied between -0.54 and -0.18. This last value corresponds to a situation where an increase of 1% in the oil content leads to a decrease of only 0.18% in protein (Silva, 1991; Martins and Silva, 1994).

Alkaloids are also important seed components, with an average value of about 3%, ranging from 0.01% or less up to 4% (Gross, 1982; Muzquiz, 1992). These alkaloids have an important role in

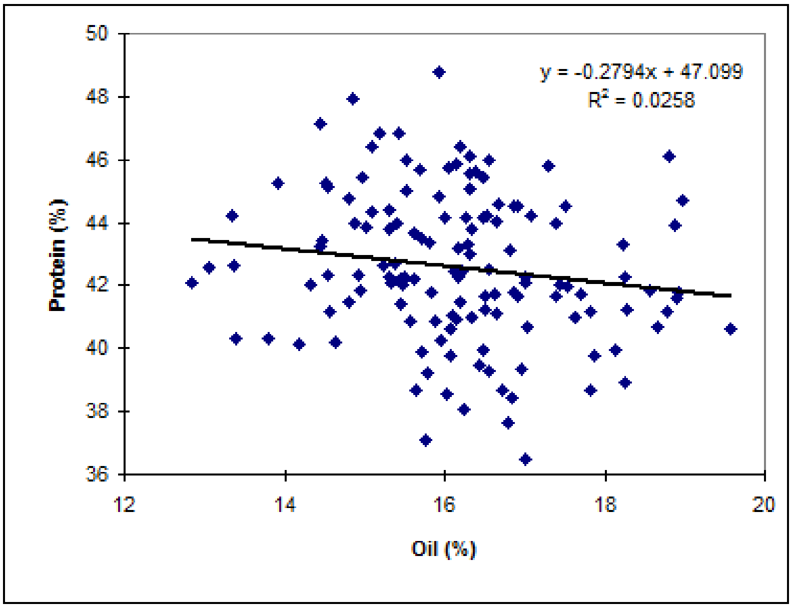

Figure 3 - Correlation between *Lupinus mutabilis* seed protein and oil content in 149 accessions.

plant protection from pests and diseases, but they difficult its use in human or animal nutrition, needing to be removed industrially (Carvajal-Larenas *et al*., 2016). Some sweet mutants were obtained, as those carrying the gene *mutal* (0.2 to 0.3% alkaloids; Williams *et al*., 1984) and the variety 'Inti' (only 0.0026% alkaloids; von Baer, 1986).

The remaining seed components are carbohydrates (15.9 to 20.5%) and cellulose in the hull (about 10%) (Gross, 1982; Carvajal-Larenas *et al*., 2016). Besides alkaloids, tarwi seeds contain no other antinutritional factors, like protease inhibitors, hemagglutinins or cyanogenic glycosides (Gross, 1982).

PRODUCTIVITY

Productivity of tarwi is low in general, but values over 2 t/ha can be obtained, depending on favourable environmental conditions (Talhinhas *et al*., 1999). In a 3-years trial with 11 accessions conducted in Lisbon, line LM231 reached the highest average yield (1.7 t/ha) while line Inti the lowest (0.9 t/ha) (Talhinhas *et al*., 1999).

There is an important relation between yield and total rainfall during the growing season. This way, both low and excessive rainfall reduces productivity. The relation between production and soil water content is better explained when yield is related with the number of days during the growing season with soil water content ranging from 30 to 95% of its capacity (r^2=0.8232) (Talhinhas *et al*., 1999).

Yield components

Due to its indeterminate growth, lupins produce seeds in the main stem, in primary branches and in subsidiary branches. In a study conducted on 11 tarwi accessions (Talhinhas *et al*., 1999), in average, while the main stem only accounted for about 30% of the total number of pod sets, it produced 63% of seed weight (Table 1).

Also, seeds tend be smaller in higher level branches. The main stem and the primary branches were the place where most production was obtained (95%) and so they are the main aim for the breeder who wants to improve seed yield. Nevertheless, superior order branches can be important to have a more stable productivity in years where environmental conditions (mainly cold days in Spring) damage main stem and primary branches productivity. These observations are reinforced knowing that yield is correlated with varying morphological characteristics according to sowing dates (Talhinhas *et al*., 1999). In fact, the yield of October-sown accessions depends on morphological

Table 1 - Yield components of *Lupinus mutabilis* derived from the average of an 11 accessions field trial conducted in Lisbon.

	Main stem	Primary branches	Secondary branches	Tertiary branches
Nr. pod sets/plant	1.0	1.8	0.5	0.8
Nr. pods/pod set	9.2	3.8	2.5	2.6
Nr. seeds/pod	3.0	2.0	1.3	0.5
Thousand seeds weight	192	171	164	153

characters related with the whole plant, while the yield of December-sown accessions depends on main stem and primary branches characters.

Comparison with other countries

According to an 11 accessions field trial comparison conducted simultaneously in Portugal (Lisbon), France (Lusignan, Poitou-Charentes), United Kingdom (Reading, Berkshire), Germany (Rastatt, Baden-Württemberg) and Poland (Wrocław, Lower Silesia) (Figure 4) tarwi productivity in the Portuguese environment tends to be higher than in Central European conditions. Results denote a clear cline of variation, with yield decreasing from Southwest to Northeast. Due to their indeterminate growth, these plants presented difficulty in maturation in Central Europe, hampering harvest (Hardy *et al*., 1998). Moreover, such studies depicted a strong genotype x location interaction, with line LM231 attaining the highest in Portugal but the lowest in the other countries, while one of the less productive lines in Portugal (LM34) was the best in Poland (Hardy *et al*., 1998).

MAIN BREEDING OBJECTIVES FOR THE MEDITERRANEAN AREA

While tarwi presents promising traits suggesting its potential as a crop for the Mediterranean area, several challenges are pose to breeding before this potential can be delivered into practice:

- Frost and cold resistance. Paradoxically, this is a specific problem for Mediterranean area, since in Central Europe tarwi is sown in Spring, while in Southern Europe plants spend Winter on the field. This way, it becomes one of the most important objectives for the breeding of this species, in order to be able to grow these plants in more continental areas of the Mediterranean.

- Precocity. This is an important problem for Central Europe, since it is necessary to adapt the typically long lupin growth cycle to the short period of Spring/Summer of these areas, being necessary a plant with an early flowering and an early maturity. In the Mediterranean area, an early flowering plant can be quite damaged by late frosts, but then it is necessary a fast pod filling (early maturity) so that the plant can produce the maximum before the end of the growth cycle.

- Architecture. For unstable climates, such as the Mediterranean one, plants with an indeterminate growth are advisable in order to stabilise yield throughout the years, even if with some losses in potential yield because of a more important competition for the photoassimilates between filling pods and growing branches.

- Productivity. As in any other crop, neglected species are likely to provide gains in breeding for yield with relative ease as compared to main crops where breeding has already stretched yield gains to high levels. Yet, this breeding must be based in locally adapted plants.

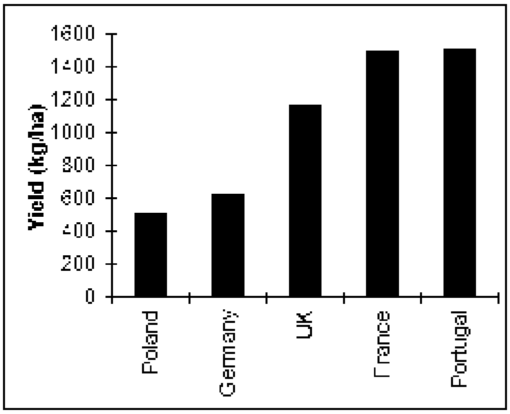

Figure 4 - Average yield of 11 *Lupinus mutabilis* accessions cultivated in five European locations (Neves-Martins *et al*., 1997).

- Oil and protein. Two different objectives can be defined in what concerns seed quality. One is proposing this plant to be an oil and protein crop, and then the aim is thus to increase seed oil content without any major reduction in protein. The other would be to face this species exclusively as a protein crop. The choice between these two objectives depends on the climatic conditions of the area (considering that cold nights at maturity are necessary for oil accumulation). If the oil quality is quite good when compared with oils from other species, the same does not apply to the protein quality, where a lot of work can still be done (mainly in what concerns an increase in the rate of sulphured amino acids).

- Alkaloids. Although sweet mutants have already been identified, avoiding the need for industrial debittering, sweet varieties are more sensitive to pests and diseases. Ideally, the aim should be to obtain a "bitter-sweet" variety, with alkaloids in leaves and stems but with not in seeds.

CONCLUSIONS

Tarwi is one the richest legume crops concerning seed oil and protein content and presents excellent potential to marginal areas. Being partially domesticated and selected as a food crop for several centuries in the Andes, nevertheless this species present a rich germplasm pool ranging several climatic regions and altitudes, conveying therefore high plasticity for breeding. To accomplish the objective of fulfilling the potential of tarwi as a crop for the Mediterranean climate, breeding needs to address challenges such as cold tolerance and adaptation to a Winter-Spring growth cycle, phenotypic plasticity, yield, chemical composition and resistance to pests and diseases.

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