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IMPACT OF GRASSPEA GENOTYPES AND SOWING DATES ON SEED β-ODAP CONCENTRATION AND AGRONOMIC TRAITS

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ABSTRACT

Grasspea (*Lathyrus sativus*) is an important food legume crop in Ethiopia. However, its nutritional value is hindered by β-ODAP that causes lathyrism in humans. The extent of toxicity is influenced by genetic and agronomic factors.We conducted an experiment to determine the effect of varieties and sowing dates on the β-ODAP content of the seeds and other yield components.Two varieties, Bio-520 and landrace, were planted on July 24, August 7 and 21, and September 4 in the 2001-02 and 2002-03 cropping seasons. Grasspea variety and season, significantly influenced β-ODAP content of the seeds.The highly significant variety x season interactions suggests that varieties behave differently in different seasons for their β-ODAP content. The main effects and interactions were highly significant for days-to-flowering and plant height. Although β-ODAP content of the varieties varied with season, the improved genotype always contained less β-ODAP concentration.Thus, while the development of low-toxin grasspea lines is the primary goal, modifying agronomic practices is also important to mitigate lathyrism.

Key Words: Ethiopia, *Lathyrus sativus*, Lathyrism, neurotoxin

RÉSUMÉ

Le pois carré (*Lathyrus sativus*) est une importante légumineuse pour l'alimentation humaine en Ethiopie. Cependant, sa valeur nutritionnelle est handicapée par le β-ODAP qui cause le lathyrisme chez les humains. Le degré de toxicité est influencé par des facteurs génétiques et agronomiques. Un essai était conduit pour déterminer l'effet de variétés et de dates de plantation sur la teneur en β-ODAP dans les graines et autres composantes du rendement. Deux variétés notamment la Bio-520 et le landrace, étaient plantées pendant les saisons culturales 2001-02 et 2002-03 en Juillet 24, le 7 et le 21 Août ainsi que le 4 Septembre. Les variétés du pois carré et la saison ont significativement influencé la teneur du β-ODAP dans les graines. Les interactions hautement significatives entre variété x saison suggèrent que les variétés se comportent différemment pendant des saisons différentes en termes de la teneur en β-ODAP. Les effets principaux et interactions étaient hautement significatifs à la floraison et en fonction de la hauteur des plants. Bien que la teneur en β-ODAP variait avec la saison, les génotypes améliorés contenaient une basse concentration en β-ODAP. Ainsi, pendant que le développement du pois carré est un objectif prioritaire, la modification des pratiques agronomiques est aussi importante pour la mitigation du lathyrisme.

Mots Cles: Ethiopie, *Lathyrus sativus*, Lathyrisme, neurotoxine

INTRODUCTION

Of the many challenges facing mankind today, achieving food security and adequate nutrition for the world's burgeoning population is the most daunting (Godfray *et al*., 2010), especially as world food production is likely to be hampered by the negative effects of climate variability and change (IPPC, 2008). This enormous task will require re-assessment of traditional approaches and the development of new ideas and strategies (Federoff *et al*., 2010). In this process, we must recognise areas of the world where there are limitations to land expansion, soil quality, and water resources. Such conditions prevail in the Mediterranean area (Khouri *et al*., 2011), extending to other semi-arid areas of the developing world.

While world food production is largely driven by major nutrients supplied by commercial fertilisers (Stewart *et al*., 2005) as well as micronutrients that impact nutrition as well as crop yield (Stein, 2010), less emphasis has been given to minor crops, and to the alleviation of anti-nutritional factors. Grasspea (*Lathyrus sativus*) is a novel crop of significance in relatively dry areas, but its use is constrained by an anti-nutritional factor or toxin, (3-(N-oxalyl)-L-2, 3-diamino propionic acid (β-ODAP), that is found in the seeds and seedlings. The toxin content can range from 0.5 to 2.5% in traditional grasspea varieties (Kumar *et al*., 2011).

Grasspea as a food/forage legume has been cultivated for millennia in Ethiopia, India, China, Bangladesh, and Nepal (Bell, 1989) and to a minor extent in southern Europe, West Asia, North Africa, and South America. It is grown as a forage crop in the Mediterranean area of western Australia (Siddique *et al*.,1996), Europe (Piergiovanni *et al*., 2011) and North America (Campbell *et al*., 1994), where it is viewed as an N-fixing crop under cereal production (Rao and Northup, 2011). As grasspea is adapted to semi-arid conditions, where much of the world's poor live, it is often regarded as a food of last resort in times of famine.

Attempts to solve the problem of the toxin β-ODAP have mainly centred on breeding of lower β-ODAP in the seeds and the forage (Kumar *et al*., 2011; Siddique*et al*., 2006). Other factors such as available soil zinc have shown to reduce the β-ODAP concentration (Lambein *et al*., 1994; Abd El-Moneim *et al*., 2010), although the mechanism involved is unclear. There is considerable evidence that expression of the toxin level of any grasspea variety is influenced by edaphic factors such as temperature and rainfall, and, thus, is location-specific (Fikre *et al*., 2011; Kumar *et al*., 2011; Jiao *et al*., 2011).The ultimate approach to β-ODAP detoxification involves boiling the seed prior to human (Mikiè *et al*., 2011) or animal consumption (Tadelle *et al*., 2003).

Because of its arid environment and the frequency of famine, much of the literature on grasspea emanated from Ethiopia (Kumar *et al*., 2011), where its adaptation is attributed to disease and insect resistance as well as drought tolerance (McCutchan, 2003), making it an insurance crop in times of famine (Haileyesus, 2001). Currently, the area planted to grasspea is about 150,000 ha, and it can yield up to 5 t ha^{-1} in favourable years. However, where the grasspea seed is eaten in considerable quantities for several months, neuro-lathyrism or paralysis of the lower limbs is common (Lambein *et al*., 2007). High incidences of this crippling condition are common in Ethiopia (Redda *et al*., 2005; Dadi *et al*., 2003; Haileyesus, 2001). Assessment of gasspea genotypes led to the conclusion that only β-ODAP concentrations less than 0.2% are suitable for human consumptions (Abd El-Moneim *et al*., 2001).

Collaboration between Ethiopia and the International Centre for Agricultural Research in the Dry Areas (ICARDA) focused on reducing the β-ODAP in otherwise acceptable grasspea varieties in the breeding programme. We assessed an ICARDA-bred variety and a local landrace at one of the main agricultural centers in Ethiopia for two growing seasons and considered the level of β-ODAP in the seeds as well as other compatible agronomic factors.

MATERIALS AND METHODS

This field trial was conducted for two seasons (2001/02 and 2002/03) at the Debre Zeit Agricultural Research Centre (Latitude: 10° 34' 60 N, Longitude: 35° 47' 60 E) in central Ethiopia. The soil type at the station is a deep heavy, black clay (vertisol), common in much of the highlands

(Tekalign *et al*., 2002). The long term (1953-2003) average rainfall and temperature recorded were 839 mm and 18.5°C, respectively. The agro-ecology is tepid to cool sub-moist with dry (October to May) and rainy (June-September) seasons.

Two varieties of grasspea were used; "Bio-520", a line developed in Syria at ICARDA and considered low in β-ODAP by comparison with other lines or varieties; and a local grasspea landrace that is popular with local farmers ("Ada'a local"). Both varieties were space-planted by hand at four different dates with about 2 weeks intervals in both years (July 24, August 7, August 21, and September 4). Each treatment (dates and varieties) was assigned to plots of 2 m x 4 m with four seeding rows per plot. The spacing was 50 cm between rows and 10 cm between individual plants in the row. The study design was randomisedcomplete block design with three replications.

Each plot was assessed for number of days-to 50% flowering and for crop maturity. Plant height was recorded on five randomly selected plants in each plot. Seed yield was determined by hand-threshing of the two central rows in each plot. Following hand-threshing, 100 seed-weight was determined, with batches of 100 randomly selected seeds from each treatment lot. The seed batches were sent to the Food Legume Quality Laboratory of ICARDA (Aleppo, Syria) for analysis of the β-ODAP content following the established procedure for such analyses (Briggs *et al*., 1983). Both seed qualities and agronomic yield parameters were statistically analysed using GenStat software (Payne, 2009).

RESULTS

There were significant effects of the main factors and interactions on the independent variables (Tables 1and 2). The main factor of interest, variety, had a significant effect on β-ODAP content in seeds ($P \leq 0.05$). However, the second factor of interest, dates of planting, had no significant influence on β-ODAP. There was a significant seasonal effect on β-ODAP. The interaction between grasspea variety and season was highly significant ($P \leq 0.001$), indicating the extent to which the variety influenced seed β-ODAP was modified by the growing conditions for a particular year. There was also an interaction between cropping season and planting date ($P \leq 0.05$) on β-ODAP in grass pea seeds.

The mean β-ODAP concentration was considerably higher in the seeds of the grasspea landrace (0.300%) compared to the improved line, Bio-520 (0.228%) (Table 1). The mean effect of cropping seasons was also notable, i.e., 0.288% in 2003 *versus* 0.253% in 2002. Significant interaction effects (Table 2) showed that the relative performance of the two varieties varied between growing seasons. While the main effect of delaying the planting date was not to increase the β-ODAP concentration, differences with planting date varied between the seasons.

Sowing date had a significant effects on grain yield, days-to-flowering and plant height (Tables 3 and 4). The interaction between season and sowing date was significant for all parameters, except 100-seed weight. For seed yield, only the sowing date had a significant effect, but the interactions were significant for season x sowing date (Table 3). Overall, the entire lowest seed yield was with the earliest planting (July 24), but there were little differences between the subsequent sowing dates despite a tendency of decline. The highest seed yield was at the second sowing (August 7), with little differences between the other dates. However, the interaction with season was highly significant, with the mean values in 2001/02 being about twice that of 2002/03.

Despite the importance of yield and grain β-ODAP for grasspea, other minor agronomic traits are of some relevance. Even though the range for

TABLE 1. Influence of variety and growing season on β-ODAP in grasspea seeds

Factor	Variety		Significance ($P \leq 0.05$)
	Bio-520	Landrace	
	%		
	0.228	0.300	0.0453
Season	2002	2003	
	0.253	0.288	

TABLE 2. Significant interaction effects of varieties and sowing dates on β-ODAP in grasspea seeds

Variety	Season		Sowing date	Season	
	2002	2003		2002	2003
	%			%	
Bio-520	0.163	0.295	July 24	0.200	0.269
			August 7	0.230	0.298
Landrace	0.343	0.281	August 21	0.265	0.292
			September 4	0.315	0.293

Significance, L.S.D. ($P<0.05$): sowing date =0.0453; season x sowing date = 0.0905

TABLE 3. Significant interaction of season with varieties and sowing date from grasspea seed yield

Sowing date	Season		Mean
	2002	2003	
	Kg ha^{-1}		
July 24	895	160	528
August 7	1024	861	943
August 21	789	911	850
September 4	667	886	777
Mean	844	705	775

Significance, LSD ($P\leq0.05$): sowing date = 247; sowing date x season = 349

100-seed weight was narrow, there was an overall significant effect of variety with Bio-520 having a mean weight of 9.0 g compared to 8.8 g for the landrace. Seasonal differences were more pronounced, with values for 2002/03 (9.7 g) being much higher than 2001/02 (7.8 g). There were no interactions between the primary factors. For days-to-flowering, variety and sowing date, all interactions were significant (Table 4).

Overall, the landrace took longer to reach 50% flowering (46 days) than Bio-520 (44 days). Similarly, date of planting had an overall effect, with an increase in days -to-flowering as the planting date was delayed. As indicated by secondary and tertiary interactions, changes due to varieties were modified by season and planting date. As might be expected, all primary factors, variety, season and planning date had significant influences on plant height, a trait of limited importance unless it contributed to lodging.

DISCUSSION

The improved low β-ODAP grasspea line maintained superiority over a local Ethiopian landrace, despite the variation in the β-ODAP content induced by cropping year or planting date, both factors that reflect environmental conditions such as rainfall or soil moisture. The variation in β-ODAP between the two seasons where the rain fall during October and December in 2001/02 cropping season might have improved soil moisture and less exposure to drought which is mainly responsible for increased in neurotoxin content in the seeds (Fig. 1). There was not much variation in mean monthly temperature between

TABLE 4. Influences of variety, sowing date and interactions on days-to-flowering of grasspea

Sowing date	Variety					
	Bio-520			Landrace		
	2002	2003	Mean	2002	2003	Mean
	— — Number of days — —					
July 24	40	37	38	43	37	40
August 7	41	43	42	45	43	44
August 21	50	51	51	51	52	51
September 4	32	48	40	40	48	51
Mean	41	45	44	44	43	45

Significance, LSD (P≤ 0.05): season date 1.6; variety= 1.60 = 403.4; sowing date x season= 2.3; sowing date x season variety = 3.2

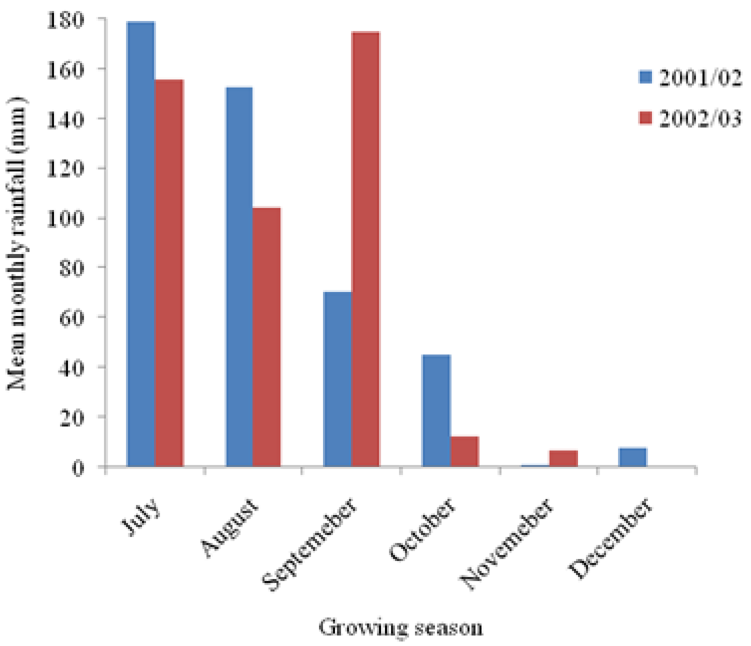

Figure 1. Mean monthly rain fall during the two cropping season, DebreZeit Agricultural Research Center, Ethiopia.

the two seasons (data not shown). Despite the significant differences between the improved line and the landrace, the range in seed β-ODAP content was small by comparison with that found in grasspea varieties in general, as reflected in studies from various areas of the world (Kumar *et al*., 2011). Given the grasspea global population of varieties, the concentration of β-ODAP in these two varieties was relatively low. Both lines had similar β-ODAP concentrations as noted by Abd El-Moneim *et al*. (2010) in a greenhouse study of three cultivars, LS 512, LS560 and LS 562. In a second greenhouse study, theses authors showed that another improved grasspea line developed by ICARDA had a β-ODAP concentration much lower (0.33%) than the improved Ethiopian landrace (0.77-0.78%). In a follow-up field study of nine grasspea accessions (Abd El-Moneim *et al*., 2010) only one had β-ODAP levels as low as the two lines in our study. Despite the relatively low β-ODAP in the developed variety, BIO-520, and the landrace, both are still acceptable for continuous human consumption based on the safe threshold value of 0.2% (Abd El-Moneim *et al*., 2001). Despite the progress that has been made in breeding to reduce the β-ODAP in grasspea grain, additional breeding efforts are needed to reduce the toxic concentrations below the critical level for use.

As with all crops, farmers who grow grasspea are interested in yield of grain or forage. There is a limit to the extent that yields can be sacrificed for quality. While there was evidence that grasspea lines low in β-ODAP have low seed yield potential (Abd El-Moneim *et al*., 2010), there were no indications of this relationship in our study. Similarly, there were no varietal differences with respect to total crop biomass yield.

Despite the limitations on β-ODAP set by the variety or cultivar, our study showed some limited possibilities for interactions by the farmer. Notwithstanding differences between seasons, the data suggest that early sowing, within the normal planting season, can have a modest effect in reducing β-ODAP in the grain, without any marked reduction in yield components.

CONCLUSION

There are advantages of an improved grasspea variety over a landrace with respect to β-ODAP concentration. While difference between seasons and planting dates are evident, more detailed and longer term studies are needed to identify and quantify the environmental factors associated with such variations. While farmers in any given location have little opportunity, other than manipulating planting date, to influence β-ODAP concentrations, the quest to greatly reduce or eliminate the toxin by conventional breeding or use of biotechnological approaches has to continue in order for this niche crop to have a role in semi-arid areas such as Ethiopia.

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