

A SENSITIVE AGAR PLATE ASSAY FOR SCREENING INHIBITORS OF α -AMYLASE

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ABSTRACT

A simple method for estimation of α -amylase inhibitor activity was developed and is being reported for the first time in this paper. This method was used for the qualitative screening of local common bean (*Phaseolus vulgaris* L.) selections of Kinnaur district for α -amylase inhibitor activity. Amongst seven common bean selections, Triloki cultivar had maximum α -amylase inhibitor activity. The results were authenticated by quantitative estimation involving the quantification of the reducing sugar (maltose equivalent) and similar results were found. Agar plate method involving less expensive chemicals and no sophisticated pieces of equipment can hence be used for screening of a large number of samples for the presence of α -amylase inhibitor. This has an application in testing natural resources for antidiabetic factors.

INTRODUCTION

Insect gut digestive α -amylases and proteinases play a key role in the digestion of plant starch (Kumari *et al.*, 2012) and proteins (Sharma *et al.*, 2012; Raj Deepika *et al.*, 2008). These digestive inhibitors provide resistance to crop plants against pests by interfering with their digestion/reproduction which causes moderate mortality, prolonged larval developmental time, and reduced fecundity (Raj Deepika *et al.*, 2008; Kumari, *et al.*, 2017; Rani *et al.*, 2018). α -amylase inhibitors of local common bean cultivar Baspa can impede the activity of α -amylases found mainly in insects (Kumari *et al.*, 2012; Rani *et al.*, 2018). Lectin-like inhibitors (α -amylase inhibitor 1 and α -amylase inhibitor-2) having inhibitory activity against mammalian and several insect amylases have been previously reported in common white, red, and black kidney beans (Kluh *et al.*, 2005). Expression of α -Amylase inhibitor 1 with the seed-specific promoter of phytohaemagglutinin from *Phaseolus vulgaris* in *Coffea arabica* had inhibitory activity on digestive amylases of *Hypotememus hampei* (Barbosa *et al.*, 2010). Prospective amylase inhibitors can be used as a biotechnological tool for the discovery of novel bioinsecticides or in the construction of transgenic plants with enhanced resistance towards pests and pathogens

Human α -amylase (α -1-4-glucan-4-glucanohydrolases) is a product of the pancreas and salivary gland responsible for the initial hydrolysis of complex carbohydrates to a mixture of oligosaccharides and disaccharides in the intestinal mucosa. The inhibitors of α -amylases are also known as starch blockers because they inhibit the digestion of starch thereby, improving

postprandial carbohydrate tolerance in people with low glucose tolerance. These inhibitors could be useful for treating obesity and diabetes mellitus a metabolic disorder characterized by chronic hyperglycemia resulting from defects in insulin secretion (Ali *et al.*, 2006). Inhibitory effect of crude α -amylase inhibitor of *P. vulgaris* cultivar Baspa has been reported against human salivary amylase and porcine pancreatic amylase (Thakur *et al.*, 2015). Purified α -amylase inhibitor protein from *Colocasia* tubers also inhibited human salivary α -amylase (Kumari *et al.*, 2012). Traditionally certain plants are used in the treatment of diabetes, particularly in developing countries where most people have limited resources and do not have access to modern treatment (Oyedemi *et al.*, 2017). Common bean (*Phaseolus vulgaris* L.) is one of the most consumed legumes worldwide and its protein is a good source of bioactive proteins. Keeping in view the potential benefits of inhibitors of α -amylase, the present studies aims at developing a simple sensitive agar plate assay for qualitative screening of a large number of cultivars/varieties for α -amylase inhibitor activity.

MATERIALS AND METHODS

Seed flour extracts of seven bean selections (*Phaseolus vulgaris* L.) viz., Baspa, Contender, Luxmi, Contender (Red), Capsule, Kentucky Wonder, and Triloki were procured from Vegetables Research Station Kalpa, Kinnaur, Himachal Pradesh, India and screened for the presence of α -amylase inhibitor activity. The extracts of seed flour (1:5, w/v) was prepared in extraction buffer as described by Gupta *et al.* (2014).

The qualitative screening of seed flour extracts of local bean cultivars was done using the agar plate method developed in our laboratory. The Agar plates were prepared by dissolving starch (1.0 g) and agar (1.25 g) in the extraction buffer followed by boiling to melt agar. The solution was then poured in Petri plates and wells were made after solidification of the gel. Porcine pancreatic α -amylase was incubated with different concentrations of crude inhibitor protein for 5 minutes at 30°C and poured into the wells. In control, the enzyme was mixed with an equal volume of 50 mM sodium glycerophosphate buffer (pH 6.9) containing 3mM calcium chloride and poured into the central well. After 4 hours of incubation, the staining of the Petri plates was done with an iodine solution. A clear zone of inhibition of α -amylase activity was measured in (cm) in treatments and compared with control (untreated porcine pancreatic α -amylase). The experiments were done in three replications with duplicates.

RESULTS AND DISCUSSION

The zone of inhibition of α -amylase activity in the presence of inhibitor was found to be maximum (91.00%) in Triloki and minimum in Kentucky Wonder (39.84%) selection. The results obtained are shown in Table 1 and Figure 1. This is the first report on the use of agar plate assay for the detection of α -amylase inhibitor activity. It was found to be suitable for

screening a large number of cultivars/varieties for the qualitative detection of α -amylase inhibitor activity. The results obtained by the agar plate assay method were confirmed by quantitative estimation of decline in activity of amylase in the presence of inhibitor protein of seed flour extracts of local bean selections as described by Gupta *et al.* (2014). The results indicated significant differences in inhibitor activity among local seed flour extracts of common bean selections against porcine pancreatic α -amylase in the presence of inhibitor protein by all the common bean selections. Maximum total α -amylase inhibitor activity was found in Triloki selection (Rani *et al.*, 2018).

Quantitative estimation of α -amylase inhibitor activity involves calorimetric estimation of reducing sugar (maltose equivalent) under assay condition using 3, 5 dinitro salicylic acid (DNSA) (Bernfeld, 1955). The enzyme inhibitory activity is expressed as a decrease in units of maltose liberated (Gupta *et al.*, 2014). This method has been routinely used by researchers to date for the estimation of alpha-amylase inhibitor activity, although it is cumbersome and requires expensive chemicals and equipment.

The present studies describe the quick estimation of the presence of α -amylase inhibitor. It is an inexpensive method, simple and does not require any expensive equipment. α -Amylase inhibitors from natural sources are important for

Table 1: Screening of local common bean (*Phaseolus vulgaris* L.) selections for α -amylase inhibitor activity by agar plate method

Local bean selections	Crude inhibitor protein (100 mg seed flour in 1 ml)					Percent inhibition				
	Percent activity of control α -amylase					volumes (ml) of crude extract				
	0.05	0.10	0.15	0.20	0.25	0.05	0.10	0.15	0.20	0.25
Triloki	33.20	33.10	33.00	16.10	9.00	66.80	66.90	67.00	83.90	91.00
Contender (Red)	66.66	60.66	51.60	50.00	33.02	33.34	33.49	49.39	50.00	67.70
Contender	66.66	66.51	50.10	50.00	32.30	33.31	39.34	48.41	50.00	66.00
Capsule	66.67	55.00	50.00	35.10	33.80	33.30	45.00	50.00	64.90	66.20
Baspa	83.33	66.56	66.50	40.00	33.32	16.67	33.44	33.50	60.00	66.68
Luxmi	84.30	68.33	53.32	50.00	40.61	15.70	31.67	46.68	50.00	59.90
Kentucky Wonder	84.33	80.10	66.66	60.00	60.15	15.67	19.90	33.30	32.03	39.84

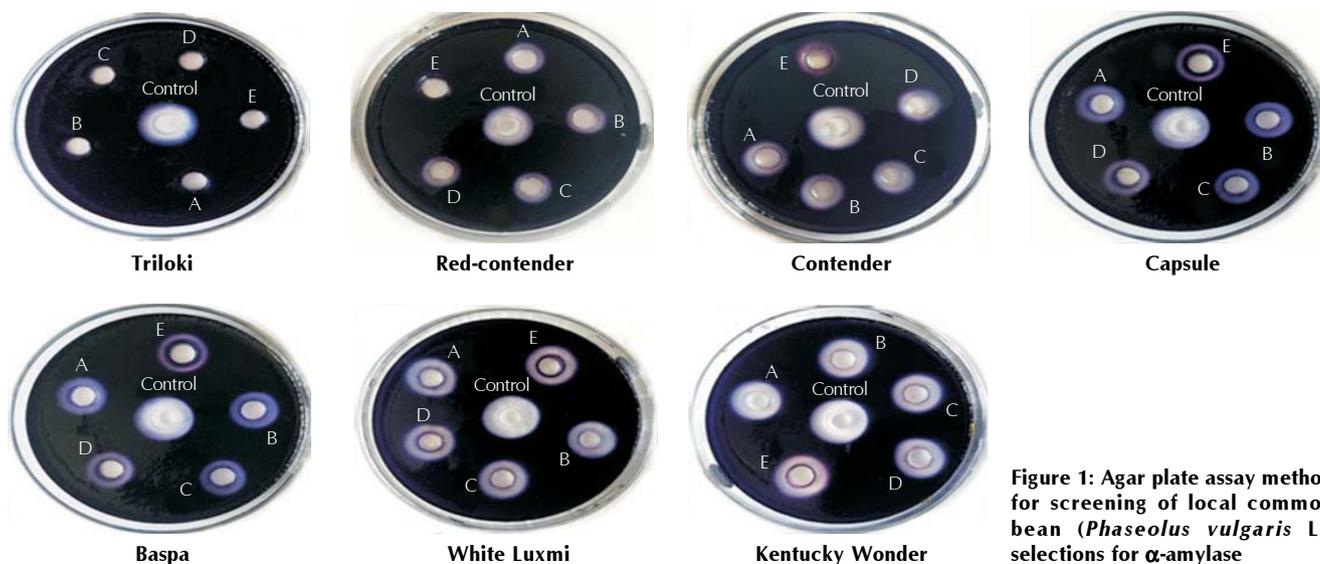


Figure 1: Agar plate assay method for screening of local common bean (*Phaseolus vulgaris* L.) selections for α -amylase

taking care of obesity and diabetes.

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