

# Comparative Investigation of Glutathione S-transferase (GST) in Different Crops and Purification of High Active GSTs from Onion (*Allium cepa* L.)

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**Abstract:** Glutathione S-transferases (GSTs; EC 2.5.1.18) are abundant proteins encoded by a highly divergent ancient gene family with protective functions through detoxification and non-catalytic roles as carriers of cytotoxins. In this work, we investigated the GST activities in seedlings of 38 crops to obtain a plant with high active GST for further study. In screening of 38 crops, onion seedling showed the highest GST activity (483.54 nmol min<sup>-1</sup>mg<sup>-1</sup> protein) followed by wheat (372.89 nmol min<sup>-1</sup>mg<sup>-1</sup> protein), barley (253.44 nmol min<sup>-1</sup>mg<sup>-1</sup> protein), rice (244.12 nmol min<sup>-1</sup>mg<sup>-1</sup> protein) and proso millet (173.34 nmol min<sup>-1</sup>mg<sup>-1</sup> protein). Carrot seedling showed the lowest activity (3.63 nmol min<sup>-1</sup>mg<sup>-1</sup> protein). In onion plants, both root and leaf showed high GST activity. Onion bulb GSTs were separated DEAE cellulose column chromatography, and purified by affinity chromatography (S-hexyl glutathione-agarose). Three GST peaks were found to elute at 67, 107 and 140mM of KCl gradient solution, and were named as GSTa, GSTb and GSTc. Among the three GSTs, GSTa, GSTb and GSTc contained 9.58, 61.45 and 28.97% of total activity, respectively. In purification, GSTa, GSTb and GSTc had specific activities of 9075, 17259 and 19868 nmol min<sup>-1</sup> mg<sup>-1</sup> protein, respectively, along with yield of 2.48, 3.17 and 1.28, and purification fold of 15.8, 29.9 and 34.5, respectively. The purity and molecular mass of the fraction was examined by SDS-PAGE. The silver staining of the purified GSTa, GSTb and GSTc indicated that final product of GSTb and GSTc were highly purified and migrated as a single band on SDS-PAGE with an apparent molecular mass of 27 kDa. However, GSTa eluted with Glyoxalase-I (Gly-I) and to purify GSTa, more methodological application are suggested.

**Keywords:** Comparative GST, Crops, Purification, High Active GST, Onion

## 1. Introduction

Glutathione S-transferases (GSTs; EC 2.5.1.18), a protein family of multiple function, catalyze the transfer of the tripeptide glutathione ( $\gamma$ -glutamyl-cysteinyl-glycine; GSH) to a co-substrate containing a reactive electrophilic center to form a polar S-glutathionylated reaction product [1]. In plant, GSTs are potential in modifying xenobiotics by glutathionation and the reaction is rapidly induced by their substrates mostly plant herbicides [2]. GSTs play essential role in different physiological processes such as stress responses, reduction of organic hydrogen peroxides and isomerization of specific metabolites [1], binding of auxin [3], cytokinin [4], and UV light depended signal transduction [5].

These enzymes were first discovered in animals in the 1960s as a result of their importance in the metabolism and detoxification of drugs. Since that time, GST activities, or the corresponding enzymes or gene sequences, have been identified in all animals, plants and fungi analyzed [6, 7].

Due to climate change, plant frequently exposes to different environmental stresses such as salinity, drought, heat, cold, light and other adverse conditions. Under abiotic stress, different endogenous cytotoxic compounds are produced in plants and the cells are not safe until they are sequestered into vacuole [6, 8]. Under abiotic stress, Plants under oxidative stress, reactive oxygen species (ROS) are produced in both radical ( $O_2^-$ ,  $\cdot OH$ ,  $OH_2$ ) and non-radical ( $H_2O_2$ ) forms. ROS causes cellular damage to metabolic

disorders and senescence processes, structural and functional loss of cell organelles, and ultimately cause plant death [9]. GSTs have important role in protecting plant cells from toxic effect through detoxification or instead of catalyzing conjugate reactions, some GSTs may have antioxidative functions [10].

The GST family in plants is notable for its structural and functional diversity and the biochemical and physiological functions. Their classification and evolution have been reported by Mohsenzadeh *et al.* [7] and Dixon *et al.* [6]. Many of the GST members of remain to be elucidated. However, there are unexplored plant species to search new GSTs. Studied of GSTs in a large number of plant species together might provide comparative GST levels in different plants and also helps to find unexplored plant species with high active GST which might have detoxification as well as

antioxidative roles in cell. However, report on comparative GST study in wide number of crops is rare. Therefore, in this study, we compared GST activities in 38 importance crops. Here, the GSTs were purified from plant with the highest level of GST activity.

## 2. Materials and Methods

### 2.1. Plant Materials

Fifteen days old seedlings of 38 different crops (except millets, 30 days) (Table1) were grown in plastic pot under greenhouse conditions. Whole seedlings were used in comparative study of GST. For comparative GST activities in plant parts, mature onion plants were used and for purification of GSTs, onion bulbs were selected.

**Table 1.** List of crops studied for GST activities.

Sl. No.	Crop	Sl. No.	Crop
1.	Carrot ( <i>Daucus carota</i> ) advanced lineCr0021	20.	Chick pea ( <i>Cicerar ietinum</i> cv BARI Chick pea-6)
2.	Sweet potato ( <i>Ipomea batatus</i> cv BARI Sweet Potato-8)	21.	Cowpea ( <i>Vigna unguiculata</i> cv BARI Cowpea-1)
3.	Potato ( <i>Solanum tuberosum</i> cv BARI Potao-30)	22.	Lentil ( <i>Lens culinaris</i> cv BARI Lentil-2)
4.	Ginger ( <i>Zingiber officinale</i> cv BARI Ginger-1)	23.	Safflower ( <i>Carthamus tinctorius</i> cv BARI Safflower-1)
5.	Turmeric ( <i>Curcuma longa</i> cv BARI Turmeric-3)	24.	Sunflower ( <i>Helianthus annuus</i> cv BARI Sunflower-2)
6.	Bottle gourd ( <i>Lagenaria siceraria</i> cv BARI Bottle gourd-4)	25.	Ground nut ( <i>Arachis hypogaea</i> cv BARI Ground nut-8)
7.	Onion ( <i>Allium cepa</i> cv BARI Onion-4)	26.	Soybean ( <i>Glycine max</i> cv BARI Soybean-1)
8.	Garlic ( <i>Allium sativum</i> cv BARI Garlic-2)	27.	<i>Brassica juncea</i> cv BARI Sarisha-11
9.	Pumpkin ( <i>Cucurbita moschata</i> cv BARI Pumpkin-2)	28.	<i>B. napus</i> cv BARI Sarisha-13
10.	Lettuce ( <i>Lactuca sativa</i> cv BARI Lettuce-1))	29.	<i>B. rapa</i> cv BARI Sarisha-14
11.	Cauliflower ( <i>Brassica oleracea</i> var Botrytis cv Rupa)	30.	Sesame ( <i>Sesamum indicum</i> cv BARISesame-1)
12.	Capsicum ( <i>Capsicum annuum</i> cv BARI Capsicum-1)	31.	Niger ( <i>Guozotia abyssinica</i> cv Shova)
13.	Tomato ( <i>Solanum lycopersicum</i> cv BARI Tomato-8)	32.	Linseed ( <i>Linum usitatissimum</i> cv Nila)
14.	Brinjal ( <i>Solanum melongena</i> cv Kazla)	33.	Rice ( <i>Oriza sativa</i> cv BRRIDhan-47))
15.	Cabbage ( <i>Brassica oleracea</i> var. capitata cv BARI Cabbage-2)	34.	Wheat ( <i>Triticum aestivum</i> cv Prodig)
16.	Mung bean ( <i>Vigna radiate</i> cv BARI Mug-6)	35.	Maize ( <i>Zea mays</i> cv BARI Hybrid Maize-7)
17.	Pea ( <i>Pisum sativum</i> cv BARI Field pea-1)	36.	Barley ( <i>Hordeum vulgare</i> cv BARI Barley-3)
18.	Grass pea ( <i>Lathyrus sativus</i> cv BARI Grasspea-2)	37.	Foxtail millet ( <i>Setaria italica</i> cv BARI Foxtail millet-3)
19.	Black gram ( <i>Vigna mungo</i> cv BARI Blackgram-2)	38.	Proso millet ( <i>Panicum miliaceum</i> cv Tushar)

### 2.2. Preparation of Soluble Protein

Soluble proteins were extracted from different plant materials by homogenizing 5gm of sample in an equal volume of 25 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, 1% (w/v) ascorbate and 10% (w/v) glycerol with mortar and pestle. The homogenates were centrifuged at 11,500 x g for 15 min, and the supernatant was used as soluble protein solution.

### 2.3. Extraction of Crude Protein for GST Purification

One hundred and fifty gm of onion bulb tissues were homogenized in an equal volume of 25 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, 1% (w/v) ascorbate and 10% (w/v) glycerol with Warming blender. The homogenates squeezed in a nylon cloth and was centrifuged at 11,500 x g for 15 min, and the supernatant was used as soluble protein solution.

### 2.4. Purification of Glutathione S-transferase

#### 2.4.1. Anion Exchange Chromatography

Proteins were precipitated from the soluble protein by ammonium sulfate at 65% saturation from the supernatant and centrifuged at 11,500 x g for 10 minutes. The proteins were dialyzed against 10 mM Tris-HCl buffer (pH 8) containing 0.01% (w/v)  $\beta$ -mercaptoethanol and 1 mM EDTA (buffer A) overnight to completely remove low molecular inhibitors. The dialyze was applied to a column (1.77 cm i.d. x 20 cm) of DEAE-cellulose (DE-52, Whatman, UK) that had been equilibrated with buffer A and eluted with a linear gradient of 0 to 0.4 M KCl in 750 ml of buffer A. The GST activity and absorbance ( $A_{280}$ ) were taken. The high active eluted peak at around 100 mM KCl was collected for further purification.

#### 2.4.2. Hydroxylapatite Chromatography

High active GST pool GSTa was applied on a

hydroxylapatite chromatography and eluted with linear gradient of 0-20 mM Potassium phosphate (K-P) buffer. Total 70 fractions, each containing 5 ml, were collected. The GST activity and absorbance ( $A_{280}$ ) were taken.

#### 2.4.3. Affinity Chromatography

The collected sample was applied to a column (0.76 cm i.d.  $\times$  4.0 cm) of *S*-hexylglutathione-agarose that had been equilibrated with 10 mM Tris-HCl buffer (pH 8.0) containing 0.01% (v/v)  $\beta$ -mercaptoethanol (buffer B). The column was washed with buffer B containing 0.2 M KCl and eluted with buffer B containing 1.2 mM *S*-hexylglutathione. The high active protein fractions eluted with *S*-hexyl glutathione were combined and dialyzed against buffer B, and the dialyze was used as the purified GST. The activity and absorbance ( $A_{280}$ ) were taken.

#### 2.5. Enzyme Activity Assay

Glutathione *S*-transferase was assayed spectrophotometrically (Shimadzu, UV-1800) following the methods of Rohman *et al.* [11]. The reaction mixture contained 50 mM (K-P) buffer (pH 6.5), 1.5 mM GSH, 1 mM 1-chloro-2,4-dinitrobenzene (CDNB) and enzyme solution in a final volume of 0.70 ml. The enzyme reaction was initiated by the addition of CDNB, and the increase in absorbance was measured at 340 nm for 1 min. The activity was calculated using the extinction coefficient of  $9.6 \text{ mM}^{-1} \text{ cm}^{-1}$ .

Glyoxalase I (Gly-I, EC: 4.4.1.5) activity was assayed out according to Hossain and Fujita [12]. Briefly, the assay mixture contained 100 mM K-P buffer (pH 7.0), 15 mM magnesium sulphate, 0.25mM reduced glutathione, and 3.5 mM methylglyoxal (MG) in a final volume of 0.8 ml. The reaction was started by the addition of MG, and the increase in absorbance was recorded at 240 nm for 1 min. The activity was calculated using the extinction coefficient of  $3.37 \text{ mM}^{-1} \text{ cm}^{-1}$ .

#### 2.6. Protein Quantification

Protein concentration was estimated following the method of Bradford [13] using BSA as protein standard.

#### 2.7. SDS-PAGE and Silver Staining

To check the homogeneity of purified GST enzyme and to estimate its molecular mass, SDS-PAGE was done in 12.5% (w/v) gel containing 0.1% (w/v) SDS by the method of Laemmli [14] followed by silver staining.

#### 2.8. Measurement of Molecular Weight

The molecular weight was measured by gel documentation system (Alpha-Inotech).

#### 2.9. Statistical Analysis

Data of GST level were analyzed by Statistical Analysis System (SAS (Version 9.3, SAS Institute Inc, NC, USA) following Complete Randomize Design (CRD). Means were

separated by Tukey's range test and  $P < 0.05$  was considered as significant level.

### 3. Results and Discussion

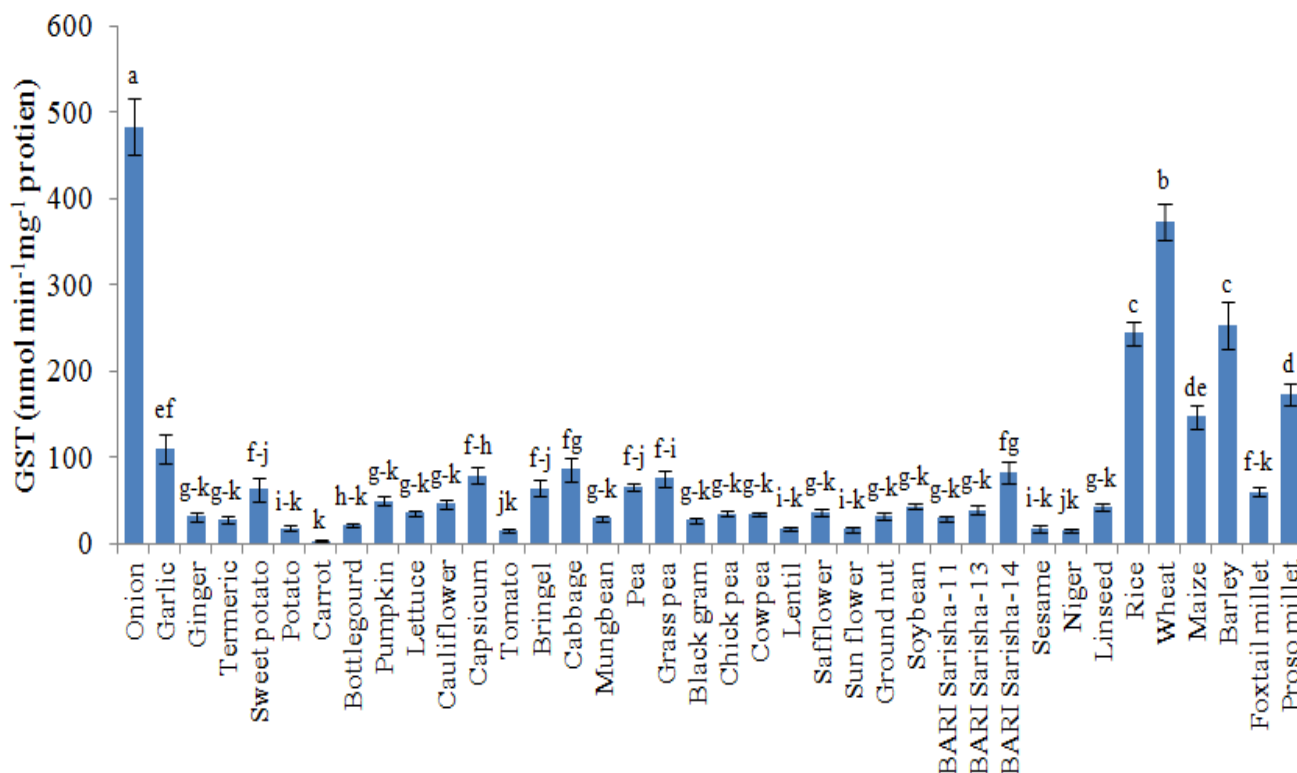
#### 3.1. Comparative Investigation of GST Activities in Different Crops

To obtain a crop with very high active GST, firstly, 38 crops were examined (Table 1). The specific activities of GST towards model substrate CDNB in the soluble protein extracts of seedlings of carrot, sweet potato, potato, ginger, turmeric, bottle gourd, onion, garlic, pumpkin, lettuce, cauliflower, capsicum, tomato, bringal, cabbage, mung bean, pea, grass pea, black gram, chick pea, cow pea, lentil, safflower, sunflower, ground nut, soybean, three rape seeds, sesame, niger, linseed, rice, wheat, maize, barely, foxtail millet and proso millet are presented in Fig. 2. The activities of GST were found in all crops and among which, onion seedlings showed the highest GST activity ( $483.54 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ) followed by wheat ( $372.89 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ), barley ( $253.44 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ), rice ( $244.12 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ), proso millet ( $173.34 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ) and maize ( $147 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ). On the contrary, carrot showed the lowest activity ( $3.63 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ) followed by tomato ( $15.01 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ), sesame ( $16.43 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ), niger ( $16.55 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ) and sunflower ( $16.85 \text{ nmol min}^{-1} \text{ mg}^{-1} \text{ protein}$ ).

Plant GSTs are mainly involved in stress responses including GSH-conjugation in the metabolic detoxification of herbicides and natural products and GSH-dependent peroxidase reactions that protect cell components from oxidative damage by scavenging toxic organic hydroperoxides. In addition, plant GSTs play a role in GSH-dependent thioltransferase that safeguards protein function from oxidative damage, and are also involved in dehydroascorbate reductase (DHAR) that functions in redox homeostasis [9]. Moreover, plant GSTs serves as ligands orbinding proteins for phytohormones (including auxins and cytokinins) or anthocyanins, thereby facilitating their transport and distribution in plants. Finally, plant GSTs play an indirect role in the regulation of apoptosis and in stress signaling pathways [1, 6, 15, 16]. Therefore, GSTs are involved in stress tolerance mechanism of plants, and are required for the characterization of plant tolerance to stresses. Previous studies have demonstrated that plant GSTs can be differentially regulated by different abiotic stress factors, such as herbicides [17, 18], hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) [19], dehydration [20, 21, 22], ultraviolet(UV) light [22], cold[18, 23, 24, 25], phosphate starvation [26], ozone exposure [27], high temperature [24, 28, 29], high salt and hormone treatments such as ethylene [30], auxin [31], methyl jasmonate, salicylic acid [32] and abscisic acid [33]. Plant GSTs play an important role in various stress responses. Recently GST activity has been reported to increase in a number of crops under drought and saline stresses [34-37]. In

this study, onion seedlings showed the highest activity (Fig. 1). Onion GSTs were previously reported in Japanese onion by Rohman *et al.* [11, 38] where five GST peaks eluted from ion exchange chromatography and among them a Phi type GST was reported with its function regarding legend binding and salinity drought and cold tolerance. On the other hand, another *Allium* crop garlic has been used to study GST in animal health, however, role of garlic GST has not been studied the biological role of GST in garlic plants under normal and abiotic stresses. Similarly, ginger and turmeric have been reported to regulate GSTs in animal bodies, but information on their GST is not available. In the cereals, wheat showed the highest activity followed by barley, rice, proso millet and maize where foxtail millet showed the

minimum activity (Fig. 1). The detoxification and antioxidant activity of GSTs in barley has been reported by Rezaei *et al.* [39]. Rice GSTs have been reported with biological role in detoxification of xenobiotic and abiotic stress tolerance [40, 41] in transgenic plants. Wheat GST also reported to detoxification and antioxidant activities [17, 42-44]. On the other hand, maize GST has been extensive studied for detoxification and transportation role [42, 45]. Recently, maize GST has been reported under abiotic stress like salinity [37] drought [46], cadmium and other stress [47]. However, proso millet which showed considerable GST activity has not been reported. Since proso millet can be grown under water stress condition, its GST might play important role under drought and other abiotic stress.



**Fig. 1.** Comparative activity of GST in the seedlings of different crops towards model substrate CDNB. The bars present the mean value of three independent experiments with three replications  $\pm$  standard error. Mean values in bars with similar letter between the bars are not significantly different at  $P < 0.05$ .

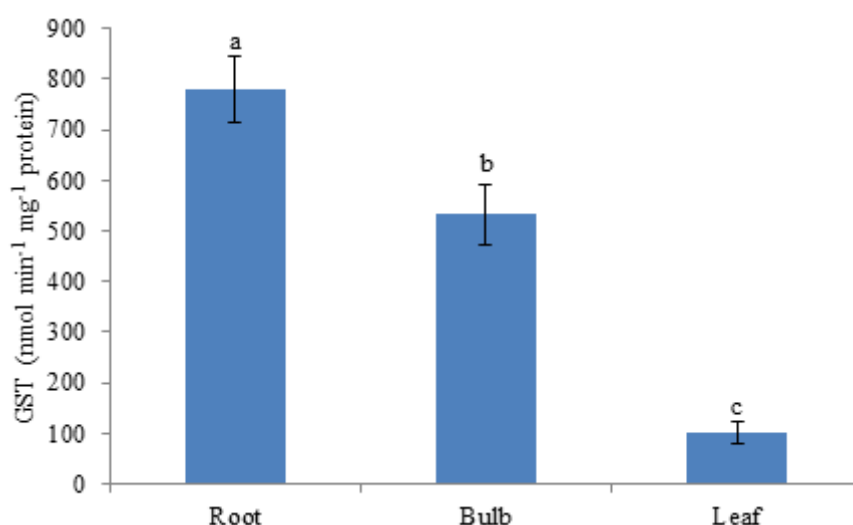
In this study, seven oil crops were incorporated among them three rape seed species viz. BARI Sarisha11, 13 and 14 from *Brassica juncea*, *B. napus* and *B. rapa*, respectively (Fig. 1). The GST activities ranged 16.43-83.17 nmol min<sup>-1</sup> mg<sup>-1</sup> protein and BARI Sarisha-14 belonging to *Brassica rapa* the highest GST activity (83.16 nmol min<sup>-1</sup> mg<sup>-1</sup> protein). GST activity has been reported to increase under biotic and abiotic stress in *Brassica* spp [48, 49], in safflower [50], in soybean [51, 52]. Role of GST also reported for root nodule formation in soybean [53]. On the other hand role of GST has been reported in sunflower under biotic stress (However, role of GST has not yet been reported for oil crop like sunflower sesame, linseed and niger though, they contains some medical values.. Therefore, some of them might have GST with important biological role under normal

and abiotic stress condition. The GST activities in pulse crops range 18.04-75.93 nmol min<sup>-1</sup> mg<sup>-1</sup> protein) and among them grass pea showed the highest activity 75.93 nmol min<sup>-1</sup> mg<sup>-1</sup> protein followed by pea (66.19 nmol min<sup>-1</sup> mg<sup>-1</sup> protein) and interestingly induced GST activities have been reported in both the crops under Cd stress [54, 55]. On the other hand, GST activity in mung bean has been studied in different abiotic stress [56, 57]. Molla *et al.* [58] compared GST activities of some lentil and mung beans, where they found higher GST activity in some lentil cultivars, where they found higher GST activity in lentil. In this study, mung bean seedlings showed higher activity compared to lentil seedlings. It might be due to using different variety. A cow pea GST has been reported to be responsive under drought stress [59]. However, report on GST in Chick pea, black gram and pea

has been not found. All of the vegetable crops showed GST activities ranged 3.63-85.98 nmol min<sup>-1</sup> mg<sup>-1</sup> protein, among which carrot had the least activity (Fig. 1). Among the vegetable crops studied, pumpkin GSTs have been extensively studied and three GSTs have been reported with their biological role [60, 61]. Among other crops, report on a potato GST [62] and tomato GST [63, 64] were found. On the other hand, the activities of cabbage under molybdenum [65] and sweet potato GSTs with trypsin inhibitor [66] were reported to be regulated. Nevertheless, reports on GST in carrot, bottle gourd, lettuce, cauliflower, capsicum and brinjal are not available, though many of them are used to regulate GSTs in animals. Therefore, they might contain GSTs with important interaction with the compound(s) present in respective crops as well as important physiological roles under abiotic and biotic stress.

### 3.2. GST Activity in Different Parts of Onion Plant

Since onion seedlings exhibited the highest GST activity, an attempt was taken to purify GSTs from onion. Before that, GST activities were examined in different parts of onion plants, and it was observed that onion root showed the highest activity (781 nmol min<sup>-1</sup>mg<sup>-1</sup> protein) followed by bulb (532 nmol min<sup>-1</sup>mg<sup>-1</sup> protein) (Fig. 2). The leaves of the seedlings had the lowest activity (nmol min<sup>-1</sup>mg<sup>-1</sup> protein). It might be due to presence of photosynthetic protein in leaves. On the other hand, the highest activity in root is not clear, and it might be due to growing environment [67]. The high GST activity in bulb is still ambiguous. However, one of the causes might be due to its regulation of physiological substrates [38]. However, due to easy availability, onion bulb was selected as plant material for purification of GST.

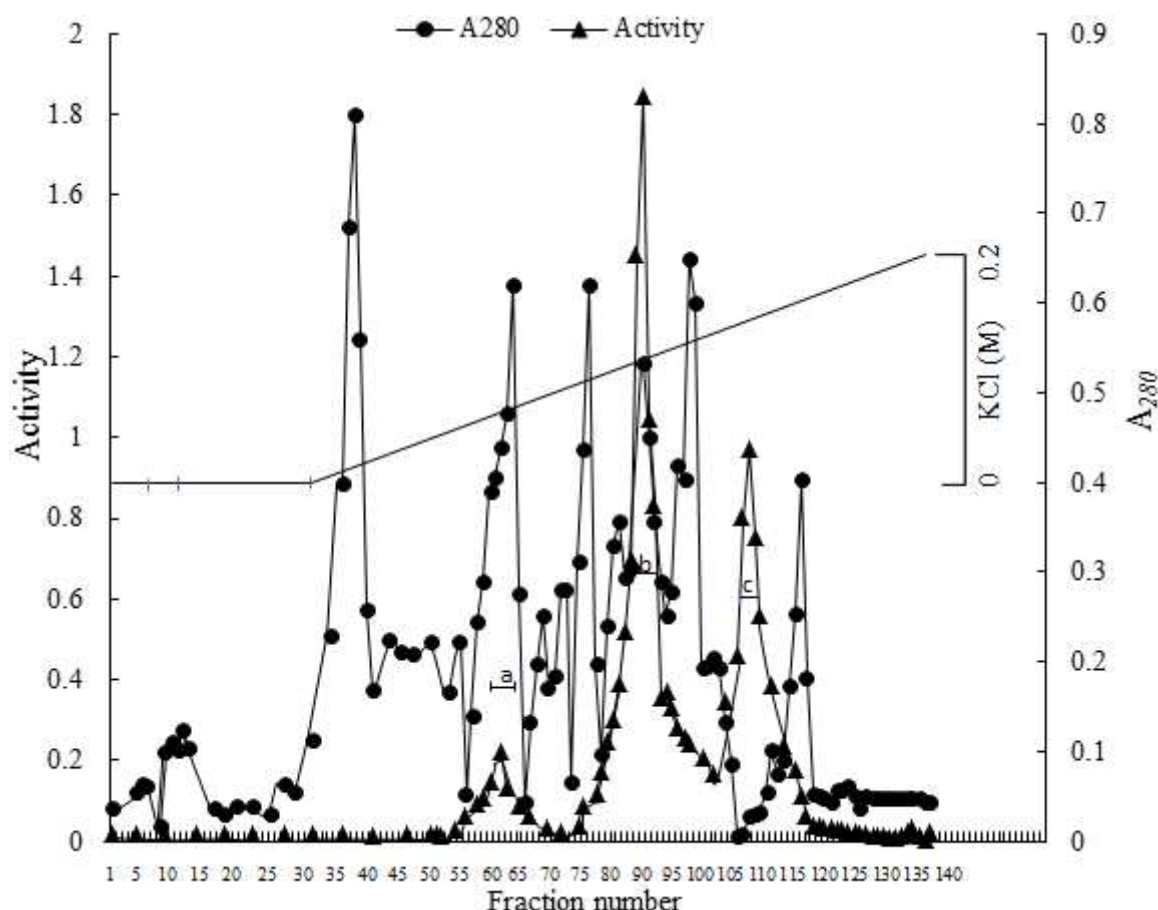


**Fig. 2.** GST activities in different parts of onion seedlings. The bars present the mean value of three independent experiments  $\pm$  standard error. Mean values in bars with similar letter between the bars are not significantly different at  $P < 0.05$ .

### 3.3. Purification of Onion GST

Cloning and characterization of plant GSTs may be helpful in knowing the stress tolerance mechanism and improvement of plant stress tolerance by molecular breeding approach. As first step of cloning and characterization, this study separated and purified GSTs from onion bulb tissues. Soluble protein were extracted from 150 g onion bulb tissue and protein was precipitated with 65% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and dialyzed over night in

buffer A. The dialyze was applied on DEAE-cellulose column chromatography (i.d. 1.7  $\times$  20 cm) and eluted with a liner gradient of KCl (0-0.2 M) (Fig. 3). Total 140 fractions, each containing 5 ml, were collected. The GST activities of each fraction towards model substrate CDNB and absorbance at 280 nm were measured. Three GST peaks eluted at 67, 104 and 140 mM of KCl were named as GSTa, GSTb and GSTc, respectively (Fig. 3 and Table 2).



**Fig. 3.** A typical column chromatography of DEAE-cellulose of soluble proteins prepared from 150 g fresh onion bulb. For each fraction, absorbance at 280 nm (●) and GST activity toward CDNB were determined. Activity (▲) is expressed as  $\mu\text{mol min}^{-1} \text{ml}^{-1}$ . Bars indicate the high active peak fractions of three onion GSTs. The fractions under the bar of GSTa, GSTb, GSTc peak were pooled for subsequent purification. The curve shows the concentration of KCl (0-0.2 M).

The high fractions containing high GST activity were collected from each peak and pooled for measuring their activities and proteins. Among them, GSTa contained 9.58% of total activity, while GSTb contain 61.45% and GSTc contained 28.97% (Table 2.).

**Table 2.** Elution pattern of component GSTs of onion bulb.

GSTs	Elution point (mM KCl)	% of total activity
GSTa	67	9.58
GSTb	107	61.45
GSTc	140	28.97

hexylglutathione-agarose to complete the purification. The GST was eluted with 1.8 mM *S*-hexylglutathione. Activities of the fractions and absorbance  $A_{280}$  were taken. The active fractions were pooled and dialyzed in B buffer overnight and subjected to measure activity and protein. The summary of the purification is shown in Table 3. It was observed that in purification, GSTa, GSTb and GSTc had specific activities of 9075, 17259 and 19868  $\text{nmol min}^{-1} \text{mg}^{-1}$  protein, respectively, along with yield 2.48, 3.17 and 1.28 and purification fold of 15.8, 29.9 and 34.5, respectively.

The active GSTs pools were applied on a column of *S*-

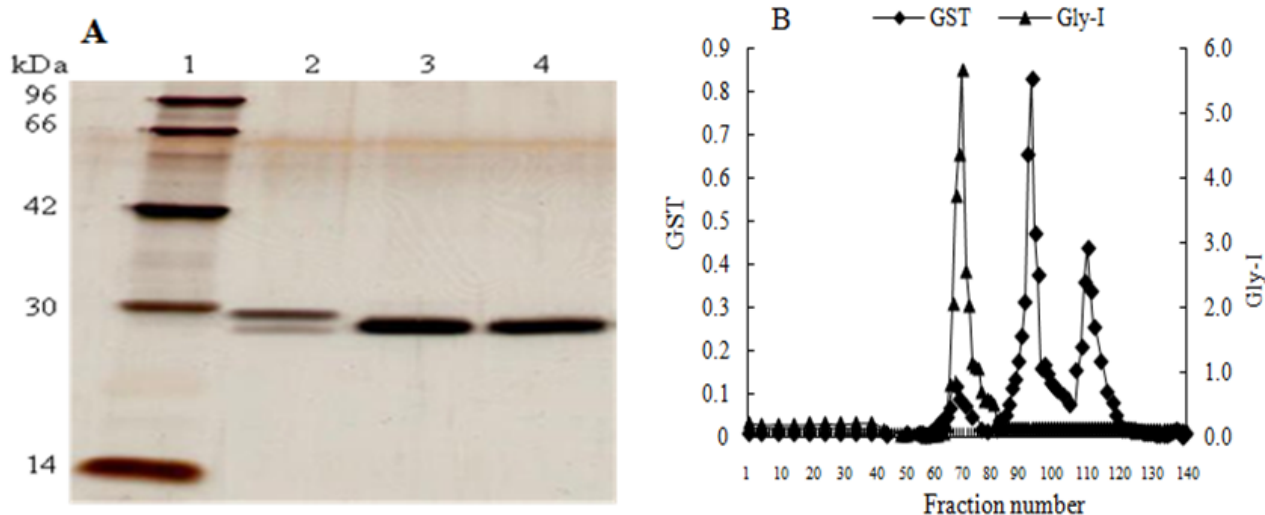
**Table 3.** Summary of purification of GSTs from onion bulb.

Fraction	Total activity ( $\text{mmol min}^{-1}$ )	Total protein (mg)	Specific activity ( $\text{nmol min}^{-1} \text{mg}^{-1}$ protein)	Yield	Purification fold
Crude protein	174.2	302.3	576	100	1.00
$(\text{NH}_4)_2\text{SO}_4$ ppt	102.3	99.8	1025	58.7	1.78
DEAE-cellulose					
GSTa	6.67	8.05	825	3.83	1.43
GSTb	42.8	5.85	7321	24.6	12.7
GSTc	20.2	4.08	4949	11.6	8.50
<i>S</i> -hexyl glutathione-agarose					
GSTa	4.81	0.53	9075	2.48	15.8
GSTb	5.52	0.32	17259	3.17	29.9
GSTc	2.38	0.12	19868	1.28	34.5



The purities and molecular masses of the purified GSTs were examined by SDS-PAGE. The silver staining of the purified GSTa, GSTb and GSTc indicated that final product of GSTb and GSTc were highly purified and migrated as a single band on SDS-PAGE with an apparent molecular mass

of 27 kDa (Fig. 4A). However, GSTa was found to have two adjacent protein bands within different molecular weight in SDS-PAGE. GSTa was further applied on a hydroxylapatite column chromatography. However, it did not produce better result.



**Fig. 4.** Silver staining of different purified GSTs purification from onion bulb (A). Lane 1, Molecular weight marker; Lane 2, GSTa; Lane 3, GSTb; Lane 4, GSTc. Elution profile of GSTs and Gly-I from affinity column chromatography (B). For each fraction, absorbance at 280 nm and GST activity ( $\blacklozenge$ ) and Gly-I activity ( $\blacktriangle$ ) were determined. Activities of GST and Gly-I are expressed as  $\mu\text{mol min}^{-1} \text{ml}^{-1}$ .

Previously, another detoxification enzyme Glyoxalase-I (Gly-I) was purified using similar chromatographies like DEAE and *S*-hexylglutathione-agarose, and in elution of Gly-I, KCl and *S*-hexylglutathione were used [12]. In this study, we also used those chromatographies as well as KCl and *S*-hexylglutathione for GST elution. Therefore, we checked the Gly-I activities in the DEAE fractions. Interestingly, a Gly-I peak eluted with similar KCl concentration where GSTa eluted, and both GST and Gly-I activities were found in same fraction (Fig. 4B). Therefore, either of the adjacent protein bands in SDS-PAGE will be a GST, and another will be Gly-I protein.

## 4. Conclusion

In conclusion, this study reported comparative GST activities in a good number of crops. The onion seedlings showed the highest GST activities as compared to others. It is also suggested that some of the crops have not yet been searched for GST which could be important sources of GST with important biological role under biotic and abiotic stresses. Local onion cultivar BARI onion-4 contained three GSTs among which, GSTb and GSTc could be purified by consecutive use of DEAE-cellulose and affinity chromatography. GSTa needed more methodological application for its purification.

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