RESEARCH NOTE

Heterosis for superoxide dismutase, peroxidase and catalase enzymes in the head of single cross-hybrids of cabbage (*Brassica oleracea* var. *capitata*)

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Introduction

Cabbage, a member of the family Brassicaceae, is an economically and nutritionally important cole crop grown in more than 90 countries and consumed widely. There are various biotic and abiotic factors responsible for reducing the productivity as well as the quality of the head. Production of deleterious reactive oxygen species (ROS) such as singlet oxygen ($^1O_2$), superoxide ROS ($O_2^-\cdot$), hydrogen peroxide (H_2O_2), hydroxyl ion (OH^-) and free hydroxyl ROS ($OH\cdot$) is one of the chief reasons for poor productivity and quality of the harvest under stressful condition (Moller 2001; Singh *et al*. 2009). If not neutralized, the ROS damage various cells causing susceptibility to biotic and abiotic stresses, yellowing (Zhuang *et al*. 1995), wilting, ethylene synthesis and senescence (Dhindsa *et al*. 1981). The consequent premature senescence is an obstacle for plant vigour, productivity and quality. Plants, however, possess well-developed defense systems against deleterious ROS, involving both limiting their formation as well as triggering their removal.

In a cell, the enzyme superoxide dismutase (SOD) constitutes the first line of defense against ROS by catalyzing the scavenging of $O_2^-\cdot$ to H_2O_2, and is followed closely in importance by peroxidase (POX) and catalase (CAT) which are responsible for reduction of $H_2O_2\cdot$ Besides these, vitamins like beta carotene (vitamin A precursor), vitamin C, vitamin E, etc.; phytochemicals such as phenol, flavonoid, phenylopronoid, etc.; and minerals like selenium and zinc are also directly or indirectly involved in defense against ROS.

McKersie (1996) classified cruciferous vegetables; namely cabbage, broccoli, brussel sprouts, collard, kale and mustard, and turnip leaves as 'super food' because of the presence of a robust oxidative defense system (antioxidants). Of different vegetable crucifers, cabbage is gaining popularity globally due to its wider adaptability, cheaper and round the year availability, and as an integral, invaluable and inseparable component of the fast food industry. Although appreciable amounts of heterosis (hybrid vigour) have been detected for horticultural traits (Prakash and Verma 2004; Xu *et al*. 2004; Singh 2007) and mineral element content (Singh *et al*. 2009), yet studies on heterosis for antioxidants in general and for the defense enzymes in particular are almost next to none. To the best of our knowledge based on available literature, there are no studies describing heterosis for antioxidant enzymes either in cabbage or any other vegetable crop. However, a few efforts have been made to enhance the antioxidant enzymes in vegetables through biotechnological means (Zambounis *et al*. 2002; Yiu and Tseng 2005). The present study tried to select hybrids possessing higher activity of the defense enzymes in cabbage through heterosis breeding, one of the most used and commercially adopted conventional breeding approaches. We examine here the magnitude and the direction of heterosis for SOD, POX and CAT activity; and identify promising parents and single cross-hybrids (SCHs) having better inbuilt defense systems which could be used for breeding antioxidant-rich cabbage.

Materials and methods

Sixteen cabbage genotypes (all inbreds) which included five female, viz. CMS-GA, Golden Acre, 83-1, 83-2 and Pride of Asia; and 11 male genotypes, viz. AC-204, EC-490174, Pusa Mukta, C-4, Red Cabbage, C-2, AC-1019, EC-490192, MR-1, AC-208 and AC-1021 comprised the basic experi-

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mental materials (table 1). The parents were chosen on the basis of their practical applicability in heterosis breeding (such as self-incompatibility and male sterility), traits of economic importance (like tolerant to diseases and insects, wide adaptability, high yield potential, uniformity in heading, etc.) and the prevalence of variability for various antioxidants. They were crossed in line × tester mating design (Kempthorne 1957) during 2005–2006 to obtain adequate amounts of F_1 hybrid seeds. The seedlings of 55 F_1 SCHs along with 16 inbreds including check variety Golden Acre were raised and transplanted in the following year at Naggar Farm of Indian Agricultural Research Institute Regional Station, Katrain, Kullu, India. The Farm is located at 32.12°N latitude and 77.13°E longitude at an altitude of 1690 m. The farm receives 110–130 cm snowfall annually. The plot size was 2.7 × 2.7 m with inter-row and intra-row spacing of 45 cm. Plot was replicated three times in a randomized block design, and the standard package of practices was followed for raising the crop.

Heads of each genotype in the replicated trial were harvested at fresh marketable stage, chopped, homogenized and a sample of 2 g fresh weight (FW) was drawn, frozen immediately in liquid nitrogen and stored at −80°C until enzymatic assay. The activity of SOD (unit of SOD min^{-1} g^{-1} FW); POX (μmol tetraguaiacol min^{-1} g^{-1} FW) and CAT (mM H_2O_2 reduced min^{-1} g^{-1} FW) was estimated using the method given by Dhindsa *et al*. (1981); Castillo *et al*. (1984) and Aebi (1984), respectively.

Analysis of variance (ANOVA) for parents and hybrids was carried out as per Kempthorne (1957), with modifications suggested by Arunachalam (1974). *Per se* performance of individual hybrid was regarded as hybrid effect. The standard error for hybrid effect and heterosis were calculated as per Singh and Chaudhary (1977). Better parent heterosis or heterobeltiosis (%) and economic heterosis (%) for each hybrid were also calculated.

Results and discussions

The mean squares due to parents, hybrids and parents versus hybrids were observed to be highly significant for all antioxidants, except parents versus hybrids for SOD, indicating the existence of sufficient amount of variation and the possibility of improvement of oxidative defense mechanisms in cabbage through population improvement and hybrid breeding. Nonsignificance of SOD mean square for parents versus hybrids is justified by meagre difference between parental and hybrids mean value (6.59 and 6.55). Six parents, namely CMS-GA, Golden Acre, Pride of Asia, Red Cabbage, MR-1 and AC-208 possessed sufficient amount of SOD, POX and CAT activity which could be exploited in further breeding programmes to develop antioxidant-rich cabbage varieties, synthetics and hybrids (table 2). This would ultimately

Table 1. Details of basic experimental materials.

		Antioxidant activity in parental line			
	Parent	Superoxide dismutase (unit of SOD min^{-1} g^{-1})	Peroxidase (μmol tetraguaiacol min^{-1} g^{-1})	Catalase (mM H_2O_2 reduced min^{-1} g^{-1})	Traits of economic importance
1	CMS-GA	7.43	1788	23.1	Male sterile
2	Golden Acre	7.10	927	90.4	Good yielder and highly adaptive
3	83-1	6.11	901	134.8	Self-incompatible
4	83-2	7.01	3966	157.8	Self-incompatible
5	Pride of Asia	7.11	2583	24.8	Highly adaptive
6	AC-204	5.92	2013	47.9	Black rot, downy mildew and diamond back moth tolerant
7	EC-490174	6.95	1816	16.0	Bigger head size and vigorous growth
8	Pusa Mukta	6.17	2285	17.8	Good yielder, highly adaptive, bumpy leaf surface and black rot tolerant
9	C-4	5.81	698	14.2	Bumpy leaf surface
10	Red Cabbage	7.09	4536	213.3	Diamond back moth tolerant, purple leaf colour
11	C-2	6.41	559	55.0	Bumpy leaf surface
12	AC-1019	6.66	1583	40.8	Uniform heading
13	EC-490192	6.60	1870	26.7	Vigorous growth
14	MR-1	7.24	1238	65.6	Black rot, downy mildew and diamond back moth tolerant
15	AC-208	7.01	1499	203.9	Black rot, downy mildew and diamond back moth tolerant
16	AC-1021	4.86	415	14.2	Uniform heading

Table 2. *Per se* performance of 55 hybrids of cabbage for SOD, POX and CAT enzymes.

Inbred	AC-204	EC-490174	Pusa Mukta	C-4	Red Cabbage	C-2	AC- 1019	EC-490192	MR-1	AC-208	AC-1021	Hybrid mean of female inbred
SOD (unit of SOD $min^{-1}g^{-1}$)												
CMS-GA	6.89	6.59	6.54	7.36*	7.93*	7.22*	5.95	5.73	7.05*	6.26	6.19	6.70
Golden Acre	6.81	7.38*	6.85	6.81	7.91*	6.64	7.00*	7.12*	6.90*	7.27*	7.00*	7.07
83-1	5.90	6.89	6.02	6.51	7.01*	5.64	6.00	5.99	7.23*	5.72	5.84	6.25
83-2	6.59	7.00*	4.65	5.86	7.22*	5.12	4.98	6.32	7.26*	6.14	6.77	6.17
Pride of Asia	6.32	5.58	6.74	7.67*	7.10*	6.79	6.81	5.15	7.49*	7.32*	5.35	6.57
Hybrid mean of male inbred	6.50	6.69	6.16	6.84	7.44	6.28	6.15	6.06	7.19	6.54	6.23	6.55
(Range, 4.65–7.93; mean, 6.55; SE, 0.18; CV(%), 4.76; CD at 5%, 0.49)												
POX (μmol tetraguaiacol min^{-1} g^{-1})												
CMS-GA	2244*	1035	1566	849	4160*	2212*	2026*	1410	2905*	1707*	946	1914
Golden Acre	663	402	588	112	1747*	490	752	546	356	1504	1261	765
83-1	860	916	821	460	741	1356	2089*	348	441	819	795	877
83-2	2199*	1167	413	1324	1154	830	1365	817	1223	853	1585	1176
Pride of Asia	462	1801*	2058*	2828*	1767*	2011*	2871*	901	2126*	2497*	1979*	1936
Hybrid mean of male inbred	1286	1064	1089	1115	1914	1380	1821	804	1410	1476	1313	1334
(Range, 348–4160; mean, 1334; SE, 134; CV(%), 17.36; CD at 5%, 371)												
CAT (mM H_2O_2 reduced min^{-1} g^{-1})												
CMS-GA	70.9	138.3*	88.7	49.6	264.2*	148.9*	163.1*	104.6	223.4*	145.4*	134.8*	139.3
Golden Acre	95.7	102.8	109.9	81.6	175.5*	88.7	138.3*	88.7	177.3*	161.3*	60.3	116.4
83-1	81.6	85.1	133.0*	74.5	101.1	101.1	124.1*	55.0	92.2	46.1	56.7	86.4
83-2	92.2	117.0*	108.2	76.2	172.0*	99.3	97.5	108.2	85.1	99.3	99.3	104.9
Pride of Asia	47.9	53.2	26.6	65.6	140.1*	19.5	205.7*	28.4	39.0	37.2	49.6	64.8
Hybrid mean of male inbred	77.7	99.3	93.3	69.5	170.6	91.5	145.7	77.0	123.4	97.9	80.1	102.4
(Range, 19.5–264.2; mean, 102.4; SE, 5.4; CV (%), 9.18; CD at 5%, 15.0. * Significant at $P < 0.05$)												

improve crop stand, tolerance to stresses, diseases and pests, and shelf life of head. It is well established that the inherent antioxidant systems would help to scavenge the detrimental effects of ROS and contribute towards stress tolerance (Bowler *et al*. 1992; Zambounis *et al*. 2002; Yiu and Tseng 2005), extension of shelf life of produce and inhibition of senescence and ageing (Zhuang *et al*. 1995; Toivonen and Sweeney 1998; She *et al*. 2003), tolerance to black rot (Gay and Tuzun 2000), etc.

The higher hybrid mean value over parent and positive heterobeltiosis mean for CAT indicate the presence of positive heterosis in general. However, the opposite result demonstrates the negative heterosis for POX and SOD activity (table 3). Desirable and significant heterobeltiosis for POX activity in only one hybrid (CMS-GA × MR-1); for SOD activity in nine hybrids (Golden Acre × Red Cabbage, Pride of Asia × C-4, CMS-GA × Red Cabbage, 83-1 × C-4, Golden Acre × EC-490 174, Pride of Asia × MR-1, etc.); and for CAT activity in 17 hybrids (CMS-GA × EC-490174, CMS-GA × AC-1021, Pride of Asia × AC-1019, CMS-GA × AC-1019, CMS-GA × EC-490192, CMS-GA × Pusa Mukta, etc.) provide an opportunity to make use of SCHs for betterment. Moreover, significant economic heterosis for SOD in 12 hybrids (CMS-GA × Red Cabbage, Golden Acre × Red Cabbage, Pride of Asia × C-4, Pride of Asia × MR-1,Golden Acre × EC-490174, CMS-GA × C-4, etc.); and in 21 hybrids each for POX (CMS-GA × Red Cabbage, CMS-GA × MR-1, Pride of Asia × AC-1019, Pride of Asia × C-4, Pride of Asia × AC-208, CMS-GA × AC-204, etc.) and CAT activity (CMS-GA × Red Cabbage, CMS-GA × MR-1, Pride of Asia × AC-1019, Golden Acre × MR-1, Golden Acre × Red Cabbage, 83-2 × Red Cabbage, etc.) indicate that most popular cultivar Golden Acre possess lower activity of these three enzymes. There is ample opportunity to improve the antioxidant content by selecting the appropriate hybrids.

The enzyme SOD catalyses the scavenging of ($O_2^-\cdot$) to H_2O_2, whereas POX and CAT are involved in reduction of H_2O_2 from plant cells. Either POX or CAT, or both, are essential to nullify the detrimental effects of H_2O_2. Therefore, it is important to select the hybrids possessing high activities of SOD, and POX and/or CAT to neutralize the total detrimental effect of $O_2^-\cdot$. Out of 55 SCHs, only eight hybrids, viz. CMS-GA × Red Cabbage, CMS-GA × C-2, Golden Acre × Red Cabbage, Golden Acre × AC-208, 83-2 × Red Cabbage, Pride of Asia × C-4, Pride of Asia × MR-1 and Pride of Asia × AC-208 were found to be superior as they exhibited higher economic heterosis for SOD, and POX and/or

Table 3. Hybrid effects, heterobeltiosis and economic heterosis for SOD, POX and CAT activity in cabbage head.

Character		SOD		POX		CAT	
Parent	Range[a]	4.86 to 7.43		415 to 4536		14.2 to 213.3	
	Mean[a]	6.59		1792		71.6	
Hybrid effects (*per se* performance of hybrids)	Range[a]	4.65 to 7.93		348 to 4160		19.5 to 264.2	
	Mean[a]	6.55		1334		102.4	
	No. of promising hybrids	20		18		17	
Heterobeltiosis	Range (%)	−33.6 to 11.4		−89.6 to 62.5		−81.7 to 499.6	
	Mean (%)	−7.1		−39.6		32.7	
	No. of promising hybrids	9		1		17	
	Promising hybrids along with heterosis (%)	Golden Acre × Red Cabbage	(11.4)	CMS-GA × MR-1	(62.5)	CMS-GA × EC-490174	(499.6)
		Pride of Asia × C-4	(7.8)			CMS-GA × AC-1021	(484.3)
		CMS-GA × Red Cabbage	(6.8)			Pride of Asia × AC-1019	(404.4)
		83-1 × C-4	(6.4)			CMS-GA × AC-1019	(300.2)
		Golden Acre × EC-490174	(3.9)			CMS-GA × EC-490192	(293.2)
		Pride of Asia × MR-1	(3.6)			CMS-GA × Pusa Mukta	(284.3)
		Pride of Asia × AC-208	(3.0)			CMS-GA × MR-1	(240.7)
Economic heterosis	Range (%)	−34.4 to 11.7		−88.0 to 352.5		−78.2 to 195.6	
	Mean (%)	−7.7		45.7		14.2	
	No. of promising hybrids	12		21		21	
	Promising hybrids along with heterosis (%)	CMS-GA × Red Cabbage	(11.7)	CMS-GA × Red Cabbage	(352.5)	CMS-GA × Red Cabbage	(195.6)
		Golden Acre × Red Cabbage	(11.4)	CMS-GA × MR-1	(220.8)	CMS-GA × MR-1	(149.4)
		Pride of Asia × C-4	(8.0)	Pride of Asia × AC-1019	(214.7)	Pride of Asia × AC-1019	(129.6)
		Pride of Asia × MR-1	(5.4)	Pride of Asia × C-4	(208.9)	Golden Acre × MR-1	(98.4)
		Golden Acre × EC-490174	(4.0)	Pride of Asia × AC-208	(174.5)	Golden Acre × Red Cabbage	(95.8)
		CMS-GA × C-4	(3.7)	CMS-GA × AC-204	(146.0)	83-2 × Red Cabbage	(91.5)
		Pride of Asia × AC-208	(3.1)	CMS-GA × C-2	(142.1)	Golden Acre × AC-208	(79.6)

[a] Unit of SOD min^{-1} g^{-1} for SOD; μmol tetraguaiacol min^{-1} g^{-1} for POX; mM H_2O_2 reduced min^{-1} g^{-1} for CAT.

CAT activity which could be utilized to improve the antioxidant activity in cabbage, especially through heterosis breeding. In this study, eventually, four hybrids, i.e. CMS-GA × C-2, Golden Acre × Red Cabbage, Pride of Asia × C-4 and Golden Acre × AC-208 were found to be most worthy as exhibited by higher magnitude of economic heterosis for antioxidant enzymes; they also possessed significant economic heterosis for net head weight, i.e. 46.9, 19.5, 14.8 and 6.6%, respectively (Singh 2007). Thus, the information generated from this study might be vital and useful for developing high antioxidant-potential hybrids to eventually enhance the plant stand in field, biotic and abiotic stress tolerance, shelf life of produce, etc. without losing the vigour advantage for yield.

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