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Heritability, Genetic Variability and Relationship among Morphological and Chemical Parameters of Strawberry Cultivars

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ABSTRACT: Two field trials of 14 strawberry cultivars were conducted at the Natural Resources and Agricultural Research Center, Kurdistan during 2008 and 2009 growing seasons. Cluster analysis grouped the 14 strawberry cultivars into 3 groups, with the highest distance between Kurdistan and Camarosa cultivars. Based on genotypic and phenotypic coefficients of variation, the highest variation of traits was recorded for stolons/plant. The berry yield had high coefficient of variability and heritability, therefore direct screening for yield would be effective for selecting promising genotypes. Berry size and berry weight showed high direct effect, and therefore, are the most important contributors of yield among morphological traits. The number of stolons/plant was the least important contributor. The principal component analysis showed that yield could have a positive effect on total soluble solids (TSS) as higher yield resulted in higher TSS.

Keywords: genotypic coefficients of variation, screening, direct effect, principal component.

INTRODUCTION

Strawberries (*Fragaria* × *ananassa* Duch.) are important fruits and excellent dietary sources due to their unique taste, flavour, ascorbic acid, potassium, fibre, other secondary metabolites and simple sugar sources of energy (Kafkas *et al.* 2007). Berry yield is a polygenic trait and greatly influenced by environmental condition. The breeders have used yield components as selection criteria to improve yield by indirect selection (Iqbal *et al.* 2009). Selection efficiency depends on the magnitude of its heritability and genetic variation for a character (Falconer, Mackay, and Frankham 1996). Information on the nature and extent of genetic variability and degree of transmission of traits is of paramount importance in enhancing selection efficiency (Ehdaie and Waines 1989). Genetic parameters such as genotypic coefficient of variability, heritability and genetic advance can provide precise estimates for genetic variation in quantitative traits (Falconer *et al.* 1996) and are used by breeders for many years to understand the genetic and environmental effects on different traits (Yap and Harvey 1972). Knowledge of the genetic association between yield and its related traits can help breeders to improve the efficiency of selection (Allard 1999). Since yield of a plant is an outcome of intricate relationships of several traits, it is imperative to identify interrelationship of traits and their direct and indirect contribution towards yield. Although the relationship between yield and other agronomic characters in many plants has been studied (Agrama 1996; Hsu and Walton 1971), such information is not frequently available for yield and other agronomic traits in strawberry.

One of the basic methods to study of the relationships between traits is analysis of correlation coefficients (Steel and Torrie 1960). Correlation between different traits with plant yield is a suitable technique to make decisions about the relative importance of these attributes and their values as selection criteria (Bramel *et al.* 1984; Saed-Moucheshi *et al.* 2013a). Principal component analysis and path coefficient analysis help to have holistic view of the relative contribution of the traits to yield (Agrama 1996; Allard 1999; Bramel *et al.* 1984; Saed-Moucheshi *et al.* 2013a).

This study was conducted to examine the relationship between berry yield and some morphological parameters to capacitate strawberry breeders for the genetic improvement of morphological and chemical traits in strawberry.

MATERIALS AND METHODS

Two field trials were conducted at the Natural Resources and Agricultural Research Center, Kurdistan, Iran during 2008-2009 growing seasons. Bartlett's test for homogeneity of variances was done for two trials (Barnett and Lewis 1994).

Experimental procedure: Fourteen cultivars of strawberry (Table 1) were planted in a Randomized Complete Block Design for two years in Kurdistan province of Iran. From each of the three replications, mean of 3 harvested samples was used for analysis. Each block was divided into 14 plots of 1m2 for planting each cultivar. Plants were irrigated twice a week.

Table 1. Strawberry cultivars used in the study and their numbers correspond in figures.

No	Cultivar name	No	Cultivar name
1	Camarosa	8	Merak
2	Classica	9	Missionary
3	Diamond	10	Pajaro
4	Fresno	11	Paros
5	Gaviota	12	Queen Eliza
6	Karcynberg	13	Selva
7	Kurdistan	14	Ventana

Data recording: Strawberries were harvested at commercially mature stage and weighed for strawberry yield per plant with an electronic digital balance (Model A&D GR-200, Made in Japan). Mean of yield, petiole length, berry weight and berry size were used for statistical analyses. Fruiting and flowering period and stolons/plant were also recorded.

The fruits of each replication were homogenized in a refrigerated blender (Model 51BL31, Torrington, CT, USA) at high speed for 2 min. The resultant homogenate was used for chemical analyses. Total soluble solids (TSS) were determined at 20°C on a Bausch & Lomb refractometer (Abbe Refractometer Model 2WAJ, Made in China). Titratable acidity (TA) was determined by diluting each 5ml aliquot of strawberry juice in 50 ml distilled water and then titrating to pH 8.2 using 0.1N NaOH (Zheng *et al.* 2007). Total anthocyanin was extracted overnight at 4°C from 1 g of homogenate with 9 ml of methanol containing 0.1% (v/v) of HCl. After centrifugation at 1500g for 10 min, the supernatant was filtered and its absorbance was measured at 510 nm. The amount of anthocyanin was calculated using the extinction coefficient (ε) equal to 3.6×10^{-6} L mol^{-1} m^{-1} (Woodward 1972). Total anthocyanin content was expressed as mg of pelargonidin-3-glucoside (PGN) per liter of liquid fruit's tissue.

Data analysis: Combined data of measured parameters over the two years of the study were used for statistical analyses. Normality tests using residuals were done and where not present, normality was gained by related transformations. Heritability, phenotypic and genotypic coefficient of variability and phenotypic and genotypic variances were derived from variance component of combined analysis of variance for measured traits based on the following formulae:

V_g = MSyg/ry, $V_P = V_g$ + MSe, GCV =

$$GCV = \frac{\sqrt{Vg}}{\bar{X}}, \quad PCV = \frac{\sqrt{Vp}}{\bar{X}}, \quad h^2 = \frac{Vg}{Vp}$$

Where abbreviations are; MSe = Error mean square, Msyg= Year × cultivar interaction mean square Vg= Genotypic variance, Vp= Phenotypic variance, GCV= Genotypic coefficient of variation, PCV= Phenotypic coefficient of variation, h2= Heritability, r= Replication, y= year and =mean of traits.

Cluster analysis was performed using a measure of similarity levels and Euclidean distance (Eisen *et al.* 1998; Everitt 1993). Principal Components Analysis was used to determine relationship among the variables (Everitt 1993). Stepwise Multiple Linear Regression procedure was used according to Draper and Smith (1966) to determine the variable accounting for the majority of total yield variability. Phenotypic correlation coefficients were calculated by the Pearson correlation method. Genotypic correlation coefficients were estimated by using variance-covariance components in multivariate analysis of variance (MANOVA). Path coefficient analysis was made on the basis of genotypic correlation coefficients using berry yield as dependent variable and the remaining estimated characters as causes. Direct and indirect effects of component characters on berry yield were worked out using path coefficient analysis (Dewey and Lu 1959). The SAS-9.2 and Minitab-16 packages were used for various analyses.

RESULTS

Genetic variability: There were significant differences among the strawberry cultivars for all the observed traits (Table 2). Effect of environment conditions (year) on TSS, berry size, stolons/plant, fruiting and flowering period was significant at $P>0.01$ or $P>0.05$ but was non-significant ($P<0.05$) for the remaining traits.

Also, year × cultivar interaction for TA, TSS, yield, petiole length, fruiting and flowering period was significant. The genotypic (GCV=67.08) and phenotypic (PCV = 72.29) coefficients of variation were the highest for anthocyanin content and the lowest (GCV = 5.81 and PCV = 15.05) for TSS. The heritability was the lowest for total anthocyanin content and the highest for stolons/plant (Table 3).

Cluster analysis: Hierarchical cluster analysis for grouping the cultivars was performed using mean value of traits (Fig. 1a). There were three clusters from the 14 cultivars, the cultivars, Camarosa, Paros, Merak, Selva, Diamond, Pajaro and Classica were grouped in cluster 1, the cultivars, Queen Eliza and Ventana were grouped in cluster 2 and finally the cultivars, Fresno, Missionary, Gaviota, Karcynberg and Kurdistan were grouped in cluster 3. The cultivars Fresno and Missionary resembled the most and cultivars Camarosa and Kurdistan had the least resemblance.

Table 2: Combined analysis of variance for measured traits during two years strawberry cultivation.

Effects	df	Mean Squares									
		Anthocyanin	Titratable acidity	Total soluble solids	Berry size	Berry weight	Berry yield	Stolons/plant	Fruiting period	Flowering period	Petiole length
Year	1	18.99^{ns}	50683^{ns}	1.23*	27.78*	22.82^{ns}	5470.15^{ns}	180.10**	242.88**	2962.201**	4.48^{ns}
Repeat(Year)	3	1310.12	13914.78	0.08	2.20	3.94	446.25	4.24	8.18	16.18454	9.24
Cultivar	13	4148.88*	77734.72**	4.56**	20.35**	16.65**	219206.36**	52.80**	65.81**	65.74075**	50.87**
Year×Cultivar	13	3714.60^{ns}	11604.51**	1.72*	3.79^{ns}	2.71^{ns}	30148.72**	3.27^{ns}	30.03**	28.95202*	22.35**
Error	52	1978.20	3737.08	0.75	2.11	1.61	4111.56	1.94	10.46	12.38179	3.43
Coefficient of variation (%)		21.22	9.58	10.66	16.21	14.91	16.80	18.16	8.54	7.1168	11.97
R^2		0.847	0.868	0.862	0.901	0.938	0.776	0.761	0.680	0.867	0.711

**, * and ns: Significant at P<0.01, significant at P<0.05 and not significant regularly. df= Degree of freedom

Table 3: Some of statistic and genetic parameters of measured traits during two years strawberry cultivation.

Traits	Mean	Min	Max	SD	SE	GV	PV	h^2%	GCV	PCV
Anthocyanin	107.9	17.53	211.25	50.34	5.49	71.38	77.58	3.53	67.08	72.29
Berry size	8.954	2.5	15	2.354	0.257	2.76	4.87	56.73	30.83	54.35
Berry weight	8.509	4.5	12.923	2.123	0.232	2.32	3.93	59.08	27.31	46.23
Flowering period	49.443	32	62	7.685	0.838	6.13	18.51	33.12	12.40	37.44
Fruiting period	37.863	29	53.795	6.912	0.754	5.96	16.42	36.31	15.75	43.37
Petiole length	15.477	3	24.67	3.757	0.41	4.75	8.18	58.06	30.70	52.88
Stolons/plant	7.669	1	15.7	3.517	0.384	8.25	10.19	80.98	67.64	82.92
Titratable acidity	638	348.2	947.2	132.7	14.5	408.70	520.80	74.68	60.14	75.67
Total soluble solids	8.138	5.4	10.3	1.214	0.132	0.47	1.22	38.60	5.81	15.05
Berry yield	381.7	52.3	1138.2	204.3	22.3	240.60	281.20	56.65	63.51	74.73

Mean: Mean of the traits; Max: Maximum value of the traits; Min: Minimum value of the traits; SD: Standard deviation for traits; SE: Standard error for mean of the traits; GV: Genotypic variation; PV: Phenotypic variation; h^2: Heritability of the traits; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation.

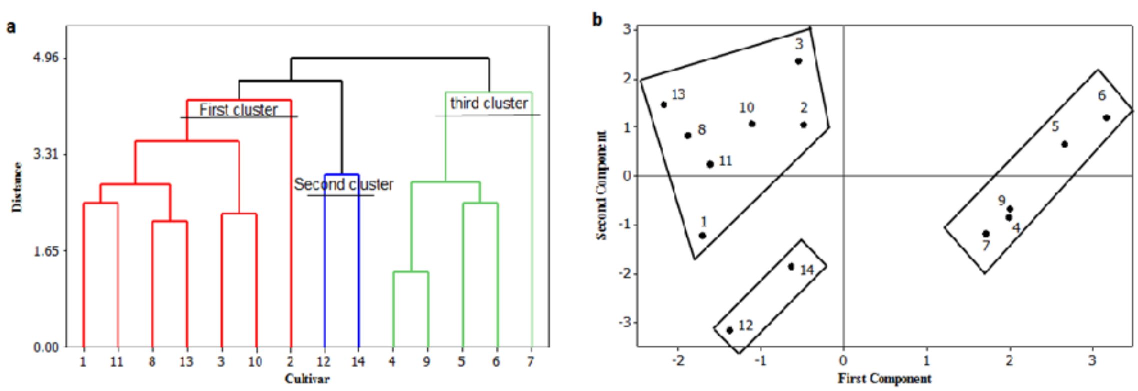

Fig. 1 (a): Cluster analyzing for grouping cultivars using two years data of measured parameters and (b): Contribution of cultivars in the first and second components of principal component analysis.

Principal component analysis: The component 1 showed the highest Eigen value (3.51) and most of the variation can be explained by this component. After component 1, the components 2, 3 and 4 can explain more variation than other components. Four first components explained 85% of the total variation (Table 4). On the other hand, these components have higher value than unit Eigen value and, therefore, these components were used for explaining the entire variation. Flowering period had the highest coefficient in component 1. In components 2, 3 and 4 the yield, anthocyanin and berry size regularly showed maximum coefficient among the traits. First and second component graphically were used for determining resemblance and distance among cultivars (Fig. 1 b).

Table 4: Principle component analysis of traits measured during two years strawberry cultivation.

	Component			
Traits	1	2	3	4
Anthocyanin	0.229	-0.191	-0.529	-0.174
Berry size	-0.379	-0.285	0.009	-0.482
Berry weight	-0.395	-0.258	0.007	-0.463
Flowering period	-0.424	-0.016	-0.342	0.326
Fruiting period	-0.383	0.012	-0.351	0.454
Petiole length	0.084	-0.48	-0.018	0.348
Stolons/plant	0.352	-0.196	-0.404	-0.107
Titratable acidity	0.025	-0.376	0.559	0.268
Total soluble solids	0.37	-0.43	-0.059	0.017
Berry yield	-0.233	-0.469	0.002	0.077
Eigenvalue	3.510	2.330	1.430	1.251
Proportion percent of variance	35.1	23.3	14.3	12.5
Cumulative percent of variance	35.1	58.4	72.7	85.2

Table 5. Results of stepwise regression in 14 strawberry cultivars during two years cultivation.

Stage	Variables entered	Variables removed	Partial R^2	Model R^2	F-value	P-value	coefficient	Standard error
1	BS	-----	0.2584	0.2584	4.18	0.0635	12.39	15.33
2	BW	-----	0.1499	0.4083	2.71	0.0973	23.08	14.9
3	FrP	-----	0.1951	0.6034	2.92	0.1181	555.66	322.88
4	PL	-----	0.0936	0.697	1.65	0.1398	542.9	289.53

BS: Berry Size; BW: Berry Weight; FrP: Fruiting Period; PL: Petiole Length

Stepwise regression: In step one of stepwise regression, berry size as the most effective contributor of berry yield was inserted to the model and in other three steps of stepwise regression, berry size, fruiting period and petiole length were inserted as well, but none of them were removed. By using these most effective contributing traits, model R-square gained to 0.70 (Table 5). The obtained results showed that the prediction equation for yield per plant (Y) using four variables that were inserted in the model by stepwise selection is formulated as follows:

$$Y= -816.39 + 12.39\ BS + 23.08\ BW - 555.66\ FrP + 542.9\ PL$$

Where the abbreviations are BS: Berry Size; BW: Berry Weight, FrP: Fruiting Period, PL: Petiole Length.

Correlation coefficients: Petiole length had a significant positive correlation with yield and TSS while there were no other significant correlations related to this trait (Table 6). Correlations between flowering period with fruiting period, stolons/plant and TSS were negatively significant but with weight and berry size were positively significant. Except negative significant correlation with flowering period, none of other fruiting period correlations was significant. Correlation of stolons/plant with berry weight and TSS were negative and significant while stolons/plant correlation with anthocyanin content was positively significant. Yield had positive correlations with most traits but these correlations were significant only for petiole length, berry weight and berry size. Berry weight and berry size had a high positive significant correlation with each other. TSS and anthocyanin content showed positive and significant correlation as well.

Path coefficient analysis: Highest direct effect of morphological parameters was due to berry weight and the lowest for flowering period. Direct effects of all morphological traits except flowering period were positive (Table 7). Berry weight showed negative indirect effect by berry size while berry size had positive indirect effect by berry weight. Except petiole length, other indirect effects of berry weight were negative.

Table 6: Genotypic and phenotypic correlation coefficient among traits using combined data of two years study.

Trait		1	2	3	4	5	6	7	8	9	10
Anthocyanin (1)	rp	1									
	rg	1									
Berry size (2)	rp	-0.08 ns	1								
	rg	0.10	1								
Berry weight (3)	rp	-0.11 ns	0.95**	1							
	rg	0.11	0.96	1							
Flowering period (4)	rp	0.02 ns	0.34**	0.37**	1						
	rg	0.11	0.21	0.30	1						
Fruiting period (5)	rp	0.01 ns	-0.07 ns	-0.05 ns	-0.22*	1					
	rg	-0.06	0.07	-0.09	-0.29	1					
Petiol length (6)	rp	0.04 ns	0.14 ns	0.10 ns	0.11ns	0.01 ns	1				
	rg	-0.01	0.25	0.20	0.09	0.07	1				
Stolons/plant (7)	rp	0.25*	-0.19 ns	-0.21*	-0.42**	0.18 ns	0.18 ns	1			
	rg	0.32	-0.22	-0.33	-0.32	0.17	0.16	1			
Titratable acidity (8)	rp	-0.06 ns	0.06 ns	0.05 ns	0.11 ns	-0.16 ns	0.21 ns	-0.24*	1		
	rg	0.14	0.18	-0.09	0.21	-0.19	0.26	-0.25	1		
Total soluble solids (9)	rp	0.27*	-0.13 ns	-0.17 ns	-0.23*	-0.07 ns	0.28*	0.42**	0.14 ns	1	
	rg	0.30	-0.16	-0.19	-0.18	-0.17	0.34	0.35	0.14	1	
Berry yield (10)	rp	-0.02 ns	0.40**	0.35**	0.10 ns	0.17 ns	0.29**	-0.10 ns	0.21*	0.20 ns	1
	rg	0.09	0.51	0.48	-0.06	0.18	0.33	0.09	0.23	0.22	1

**, * and ns: Significant at P<0.01, significant at P<0.05 and not significant regularly. rg and rp: Genotypic and phenotypic correlations.

Table 7: Path analysis consists of direct and indirect effect of morphological traits on strawberry yield.

Traits	1	2	3	4	5	6	Correlation with yield
Petiole length (1)	***0.203***	-0.015	0.012	0.03	0.166	-0.07	**0.33**
Flowering period (2)	0.018	***-0.156***	-0.053	-0.061	0.248	-0.059	**-0.06**
Fruiting period (3)	0.014	0.035	***0.182***	0.0.32	-0.075	-0.02	**0.18**
Stolons/plant(4)	0.032	0.049	0.031	***0.189***	-0.274	0.06	**0.09**
Berry Weight (5)	0.04	-0.047	-0.017	-0.063	***0.83***	-0.266	**0.48**
Berry Size (6)	0.05	-0.12	-0.14	-0.042	0.572	***0.279***	**0.51**

Bold and italic numbers on main diagonal are direct effects of parameters and other numbers are indirect effects.

Table 8: Parameters identified as crucial in strawberry yield with each one of the used statistical techniques.

Trait	correlation	Stepwise regression	Path analysis	Principal component	Total score	Effect's kind
Petiole length	No (0)	Yes (1)	No (0)	No (0)	1	Positive
Flowering period	No (0)	No (0)	No (0)	Yes (1)	1	Negative
Fruiting period	No (0)	Yes (1)	No (0)	Yes (1)	2	Negative
Stolons/plant	No (0)	No (0)	No (0)	No (0)	0	-----------
Berry Weight	Yes (1)	Yes (1)	Yes (1)	Yes (1)	4	Positive
Berry Size	Yes (1)	Yes (1)	Yes (1)	Yes (1)	4	Positive

Based on each statistical techniques, which parameter have effect (positive or negative) on berry yield have score 1 (Yes) and which have not effect have score 0 (No).

DISCUSSION

A good degree of genetic variability appeared among the strawberry cultivars for the tested traits. The phonotypic and genotypic coefficients of variability were higher for total anthocyanin content but its heritability was the lowest. Anothocyanins are antioxidant which can scavenge the reactive oxygen species (ROS). ROS cause damages to plant cells and organelles, so that higher content of anthocyanin, with ability to scavenging the ROS, is a favorite case for researchers (Saed-Moucheshi *et al*, 2013c). On the other side, the broad sense heritability is not a real estimate of the index of transmissibility and gives an idea of the degree of genetic determination of a trait (Falconer *et al.* 1996).

Heritability of total anthocyanin content was low while heritability of berry yield was high, and therefore, some preliminary guidelines could be obtained for using these traits as selection parameters. Such estimates have been previously used in many plants (Aliakbari *et al.* 2013; Ehdaie and Waines 1989; Maniee, Kahrizi, and Mohammadi 2009; Moghaddam, Ehdaie, and Waines 1997; Safavi *et al.* 2010; Yap and Harvey 1972).

Cluster analysis grouped the cultivars into three clusters and the principal component analysis confirmed the results obtained from cluster analysis (Fig. 1b). The hybridization involved distantly related parents can provide a broad spectrum of variability to ensure the efficiency of selection toward better types (Allard 1999; Saed-Moucheshi, Pessarakli, and Heidari 2013b). The highest difference or distance between cultivars was observed between Camarosa and Kurdistan and crossing between them would yield high variation. The constitution of different clusters helps in clarity on the origin and evolutionary trends as well. Cultivars in the same cluster probably are from the same or close regions. On account of close placement, the cultivars, Queen Eliza and Ventana are likely to share the proximal regions. Clustering and principal component analyses are statistical techniques the breeders use worldwide for improving crop plants (Aliakbari *et al.* 2013; Anderberg 1973; Eisen *et al.* 1998; Moucheshi, Heidari, and Dadkhodai 2011; Pakniyat, Saed-Moucheshi, and Haddadi ; Saed-Moucheshi *et al.* 2013a).

For a comprehensive understanding of relationships among traits and their effect on yield multiple statistical procedures, principal component analysis, stepwise regression, coefficient of correlation and path analysis were applied to the data. Principal component analysis showed that the four first components explained 85% of total variation among the data. First component (PC1) clearly separated the two groups of variables, the chemical and morphological traits. Yield, berry size, berry weight, flowering and fruiting periods had high and negative correlations with PC1 and though, based upon this component these traits have higher effects in contributing to yield. In PC2, petiole length, TSS and yield showed the highest and negative correlation with this component. PC2 explains that petiole length had a high effect on yield, and higher yield can provide higher amount of TSS. Berry size, berry weight and yield had a low correlation with PC3 and based on this component, these two traits can be important distributors of yield. Flowering and fruiting periods and anthocyanin content showed the highest negative contribution in PC3 and therefore, these two periods would affect the anthocyanin content. The TA had the highest positive correlation with PC3 and this trait was independent from other variables. The PC4 also showed that higher yield provided higher TSS content and direct selection for yield would result in more TSS content. Multivariate and other statistical analysis used here have been applied by researchers in wheat (Hsu and Walton 1971; Leilah and Al-Khateeb 2005; Saed-Moucheshi *et al.* 2013b), barley (Yin *et al.* 2002), corn (Jaynes *et al.* 2003), soybean (Leilah and Al-Khateeb 2005) chickpea (Moucheshi *et al.* 2011) in order to understanding the relationship among traits and impact of related variables on yield. Stepwise regression selected berry size and weight, fruiting period and petiole length as the most important factors among morphological traits in contributing to berry yield. Based upon this method, berry size, the first factor entered to the model, is the most important factor on berry yield. Fruiting period had a negative coefficient for yield, thus higher fruiting period would provide lower berry yield.

Correlation coefficient analysis showed that berry size and weight which had the highest positive correlation, showed significant positive correlation with yield, while other variables showed no significant correlation with yield. The highest direct effect was observed for berry weight. Except of petiole length, indirect effects of berry weight via other variables were negative which indicated that increasing other variables have negative effect on yield through this trait. Berry size had the second highest direct effect on berry yield and it had positive indirect effect through berry weight showing that berry size would also add to yield. Stepwise regression of fruiting period and flowering also had a negative direct effect on yield.

Overall, it could be stated that berry size and berry weight, which had a direct positive relationship, are the most important traits contributing to yield and therefore are suggested for indirect selection for affecting berry yield in Strawberries.

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