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Evaluation of Triticale (×*Triticosecale*) genotypes for determination of selection parameters, trait associations and genetic diversity using grain yield and quality traits

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ABSTRACT

The study was carried out for two consecutive winter (*rabi*) seasons i.e. 2019–20 and 2020–21 at Punjab Agricultural University, Ludhiana, Punjab to explore trait relationship and genetic divergence for yield and grain quality traits among 20 triticale (×*Triticosecale* Wittm. ex A. Camus) genotypes. The experimental trials were laid out in a randomised complete block (RCBD) design with three replications. The traits assessed were Grain yield/plot (kg), Test weight (kg/hL) (TW), 1000-grains weight (g) (TGW), Grain appearance score (GAS), Phenol reaction score (PRS) and Sedimentation value (SDS-SV) and Grain protein content (GPC in %). The results indicated significant differences among the genotypes for various traits studied. Grain yield, grain protein content, phenol reaction and sedimentation value showed high heritability and genetic advance values. Grain yield had highly significant and positive association with GPC, sedimentation and TGW. Path analysis revealed GPC, test weight and TGW as major components for grain yield. Relative genetic distances grouped genotypes into five clusters of which cluster II was largest with nine genotypes. The inter-cluster distance was found maximum between cluster I and IV whereas maximum intra-cluster distance was observed for cluster V. Test weight, TGW and GAS contributed more than 70% of the total divergence. Principal component analysis (PCA) indicated capturing of major variability by two components PC_1 and PC_2. Genotypes T3940, T3949, T3973, T3974, T3975, T3979 were the promising genotypes for future triticale improvement programme.

Keywords: Correlation, Genetic divergence, Grain quality, Path analysis, Principal component analysis

Triticale (×*Triticosecale* Wittm. ex A. Camus) is a self-pollinating species amenable for pure line selection to attain homozygosity (Lelley 2006, Randhawa *et al.* 2015). It is a synthetic amphiploid cereal produced in 1875 by pollinating wheat (AABB or AABBDD) with rye (RR) pollen (Wilson 1876, Stace 1987) and later Rimpau in 1888 developed the first viable triticale hybrid of hexaploid wheat and rye. However, the first improved commercial cultivar was released in Hungary in 1968 (Blum 2014). Triticales were classified as primary and secondary types depending on the parental species and the proportion of the progenitors' genome retained (Furman *et al.* 1997, Oettler 2005, Hao *et al.* 2013) and have ploidy levels ranging from tetraploid (2n=28) to octoploid (2n=56) among which hexaploid (2n=42=AABBRR) is the most commonly cultivated possessing better adaptation and genomic stability (Oettler *et al.* 1991, Ammar *et al.* 2004, McGoverin *et al.* 2011). The hexaploid triticale has prolific vigour, reproductive stability, adaptation to various environmental conditions, better grain quality, high productivity, nutrient-use efficiency and lysine content (Mergoum and Macpherson 2004, Myer and Barnett 2004, Dennett *et al.* 2013, Kavanagh and Hall 2015). Modern triticale cultivars have consistently shown advantages over existing cultivars under marginal land conditions (Jessop 1996, Beres *et al.* 2012, Mcleod *et al.* 2012, Liu *et al.* 2017).

Efforts for triticale improvement has been very meagre due to its low grain qualities for human consumption and high seed cost (Blount *et al.* 2017). Triticale has broader genetic resource base due to ancestry of wheat (diploid, tetraploid, and hexaploid) and rye in different forms (primary, secondary and substituted) and thus, new genetic variation can continuously be created to further enrich the genetic pool. The applications of various breeding and genetic tools to stack desirable gene combinations in the triticale is crucial for its optimum utilisation in evolving desired plant types (Sabharwal *et al.* 1995, Randhawa *et al.* 2015). The knowledge of genetic parameters, viz. genotypic and phenotypic coefficients of variations (GCV, PCV), Heritability (h^2) and Genetic advance (GA) are

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very useful for predicting genetic progress and developing efficient breeding strategies. Genetic diversity in the germplasm determines the success of crop improvement programme (Harlan 1976, Moose and Rita 2008) and continuous evaluation of this valuable diversity for target environments and traits is required to fine-tune the crop improvement approaches. Although the initial grain cultivars of Triticale were spring-type, breeding efforts were focused mainly on improving grain yield for human consumption leading to their inconsistent performance and lower popularity (Randhawa *et al.* 2015). Keeping all above in view, present study was carried out to determine selection criteria and genetic diversity for further improvement of triticale genotypes.

MATERIALS AND METHODS

Plant materials: The experimental materials in the present investigation consisted of 20 genotypes of triticale including three check cultivars (TL2942, TL2969 and TL2908). Among the test genotypes, 17 genotypes (T3868, T3895, T3901, T3902, T3937, T3938, T3940, T3949, T3956, T3973, T3974, T3975, T3977, T3978, T3979, T3983 and T3984) were advanced breeding lines selected from preliminary yield trials (PYT) planted in 2018–19 season at Punjab Agriculture University, Ludhiana (30°40’N, 74°44’E; at an elevation of 247 m amsl), Punjab whereas check cultivars were released for commercial cultivation in the north-western plains and northern hills zone of Indian wheat areas. The study was carried out for two consecutive winter (*rabi*) seasons i.e. 2019–20 and 2020–21 at Punjab Agricultural University, Ludhiana, Punjab. The experiment was laid out in a randomised complete block design (RCBD) with three replications. Each experimental plot comprised six rows of 6 m length spaced at 20 cm. The seed rate/plot was calibrated at 100 kg/ha. The fertiliser dose/plot was applied based on recommended dose of 40 kg N and 20 kg P_2O_5/ha. The recommended agronomical practices were adopted to raise a good crop.

Recording observations: As triticale is considered to be better source for grain quality traits, the observations were recorded for Grain yield/plot (kg) (GY), Test weight (kg/hL) (TW), 1000-grains weight (g) (TGW), Grain appearance score (GAS), Phenol reaction score (PRS) and Sedimentation value (SDS-SV) and Protein content (GPC in %). Among these, PRS is basically used for testing of varietal purity. It is indicative of high polyphenol oxidase activity and negatively correlated to the darkening of the whole meal dough and thus, chapatti quality. Another quality parameter SDS-SV provides information on the protein quantity and the quality of flour samples and used as a screening tool in cereal breeding as well as in milling applications. High sedimentation values are associated with stronger gluten. Both, test weight and kernel weight determine milling quality.

For data recording on grain quality traits, samples were randomly taken from the harvested seed of all the genotypes from all the replications during both the years and standard methodology was adopted in recording the data. Grain yield was recorded on per plot basis from all the replications measured in kg and averaged for each year. Test weight is the weight of a specific volumetric grain weight measured as hectolitre weight (kg/hL) indicating bulk density or plumpness of the grain. It is one of the major criteria of trade-oriented grain quality and a rough index for the flour yield. Test weight was measured by a low-cost device which weighs grain filled in volume of one hectolitre as per AACC 55–10.01. For recording data on 1000-grains weight or kernel weight, grains were randomly taken from the sample of each replication for both the years and 1000-grains were counted using electronic seed counter and weighted in grams using electronic balance. Grain appearance score (GAS) was recorded on 1–10 scale based on grain size, shape and colour and the score were assigned based on poor to score 1 and maximum score 10 indicating excellent grain appearance quality. To determine phenol reaction score (PRS), the seed samples were dipped in 1% phenol solution and kept for 2 h. Afterwards, the solution was drained, seeds were dried using filter paper and graded after complete drying on 0–10 scale based on dying intensity. Sedimentation value was determined using whole grain flour of the seed samples which measures the sedimentation value of the suspension of flour in sodium lauryl sulphate (SDS)-lactic acid solution as per the standard procedure (Dick and Quick 1983) following AACC method 56–70. Grain protein content (%) was estimated using Near Infrared Reflectance method as elaborated by Corbellini and Canevara (1994) following AACC method 39–25. This method is non-destructive method of grain protein analysis using Foss NIR System DS 2500F which uses approximately 50 g of sample in each treatment.

Statistical analysis: Data recorded on various traits were subjected to statistical analyses to estimate genetic variability, relationships and diversity among the triticale genotypes. The data were pooled for both the years for analysis of variance (Panse and Sukhatme 1967), Bartlett’s test (Snedecor and Cochran 1989), genotypic and phenotypic coefficients of variation (Burton 1952), heritability (Lush 1940, Falconer and Mackay 1996), genetic advance (Johnson *et al.* 1955), path coefficient analysis (Dewey and Lu 1959) and their categorisation in different groups (Robinson *et al.* 1949, Johnson *et al.* 1955, Sivasubramaniam and Menon 1973). The Karl Pearson’s simple correlation coefficient method was used to understand trait relationships. Principal Component Analysis (PCA) was done using XLSTAT statistical package (Version 2014.0.3) whereas Agglomerative Hierarchical Clustering (AHC) analysis using the XLSTAT statistical package (Version 2014.0.3) was conducted to evaluate pairwise genetic similarity among genotypes, based on the Jaccard similarity coefficient to compute a similarity matrix.

RESULTS AND DISCUSSION

Trait specific variability among genotypes: The analysis of variance from pooled data of two crop seasons showed significant genotypic differences for all the seven traits, indicating considerable amount of variation among the genotypes for each character. The pooled mean of traits indicated wide range among the genotypes (Table 1). The genotypes showed mean yield of 3.86 kg/plot with range of 2.89–5.06 kg/plot, 12.2% average GPC ranging from 10.91 (T 3937) to 13.15% (T 3974), 71.4 g mean test weight with range of 68.1 (T3868) to 74.6 kg/hL (T 3978), 4.8 average grain appearance score ranging from 3.90 (T3868) to 5.53 (T3940), mean phenol reaction score of 2.42 with range of 1.57 (T3973) to 3.43 (T3968), mean sedimentation value of 33.23 ml with range of 25.33 (T3868) to 40.67 mL (T3973) and 36.6 g mean 1000-grains weight with range of 31.67 (TL 2908) to 42.0 g (T 3973). Genotype T3975 (5.06 kg/plot) was the highest yielding followed by T3973 (5.03 kg/plot), T3979 (4.82 kg/plot) and T3974 (4.76 kg/plot). Genotypes T3974 (13.2%), T3975 (13.1%) and T3940 (13.0%) showed more than 13.0% GPC among the genotypes. The promising genotypes identified were T3973, T3974, T3949 and T3975 for 1000-grains weight, T3978, T3983 and T3977 for test weight, T 3940, T3984 for grain appearance score, T3868 and T3979 for low sedimentation value (<30) suitable for biscuit making and T3973, T3974 and T3956 for low PRS. Among the genotypes, T3974, T3973, T3949 and T3975 were found to be promising genotypes for multiple traits and can be utilized as donor parent for yield and quality improvement in Triticale.

Trait specific variability: Variability expressed between genetic parameters has great role in crop evolution and breeding scheme. The results showed higher magnitude of PCV than their respective GCV for all the 7 characteristics (Table 1) that indicates vital role of environmental interaction in the expression of the characters (Fantahun 2006). GCV for various traits was observed from 2.15 for test weight to 25.24 for PRS. Similarly, the PCV ranged from 3.02 (TW) to 27.16 (PRS). Medium values of PCV and GCV was observed for yield/plot (18.21, 15.52) and sedimentation value (13.07, 12.32). Other traits namely GPC, TW, GAS and TGW showed low GCV and PCV values. The existence of high GCV for PRS and moderate values for GY and SDS-SV in the present study are suggestive of their utilisation as selection criteria in triticale improvement. Heritability in broad sense were worked out for the traits under study which indicated high heritability for GPC (0.93) followed by SDS-SV (0.87), grain yield/plot (0.79) and PRS (0.75). Genetic advance as percentage of mean was found to be the highest for PRS (45.79) followed by yield (29.35), SDS-SV (20.35), GAS (12.69), 1000 grain weight (12.67), GPC (10.72) and test weight (2.97). High heritability estimates coupled with high genetic advance offers the most effective condition for selection (Larik *et al.* 2000) and therefore, yield and SDS-SV may be effective parameters for quality and yield improvement in triticale. The findings of the various parameters of genetic variability are also reported in other crops by Singh *et al.* (2007), Bind *et al.* (2015), Kumar *et al.* (2016), Dashora *et al.* (2022), Mallipatil *et al.* (2023) and Biradar *et al.* (2024).

Character association and path analysis: Correlation studies are helpful in genetic upgradation of a trait by selection of another trait (Lone *et al.* 2017). Correlation between traits (Table 2) showed significant and positive association of GY with SDS-SV (0.68), TGW (0.67), GPC (0.61) and TW (0.44) whereas, it showed significantly negative correlation with PRS (-0.68). Grain protein content was significantly associated with all the traits in positive direction, i.e. TW (0.51), GAS (0.66), SDS-SV (0.50) and TGW (0.56) except PRS (-0.67) which indicated negative significant correlation. Sedimentation value was significantly correlated with TW (0.85), GAS (0.47) and TGW (0.46). PRS has negative correlation with all the traits. These trait associations may be efficiently utilised in triticale improvement programme for high yield and better-quality traits (Royo *et al.* 1994, Dogan 2009). Path analysis (Table 2) revealed highest direct effect of TGW (0.30) followed by TW (0.13). Although PRS had high direct effect on negative direction (-0.70), it showed higher indirect effects via GPC (0.63), TW (0.25), GAS (0.49), SDS-SV (0.59) and TGW (0.38). TGW had the highest direct contribution towards GY followed by TW. Path analysis emphasised prioritisation of GPC, TW and TGW during selection in view of their high heritability coupled with high genetic advance (Ramazani *et al.* 2017).

Genetic divergence (D^2) analysis: Relative magnitude of genetic distances grouped 20 triticale germplasm lines into 5 clusters (Table 3, Fig. 1). The cluster II comprising nine germplasm lines (T3895, T3901, T3902, T3937, T3938, T3979, T3984, TL2942 and TL 3969) was the largest cluster followed by Clusters III (T3940, T3956, T3977 and T3983)

Table 1 Pooled genetic parameters for seven characters in Triticale genotypes

Genetic parameters	Mean	Range	CV	SE	GCV	PCV	h^2 (bs)	GA	GAPM
Grain protein content (%)	12.20	10.91–13.15	2.61	0.18	5.84	5.91	0.93	1.46	10.72
Test weight (kg/hL)	71.43	68.07–74.60	2.25	0.93	2.15	3.02	0.41	1.99	2.97
Grain appearance score	4.80	3.90–5.53	6.18	0.17	7.20	8.41	0.67	0.54	12.69
Phenol reaction score	2.42	1.57–3.43	10.88	0.15	25.24	27.16	0.75	1.62	45.79
Sedimentation value	33.23	25.33–40.67	6.11	1.17	12.32	13.07	0.87	7.02	20.35
1000-grains weight (g)	36.60	31.67–42.00	4.78	1.01	6.77	8.28	0.67	4.17	12.67
Grain yield/plot (kg)	3.86	2.89–5.06	9.26	0.21	15.52	18.21	0.79	1.21	29.35

GCV, Genotypic coefficients of variations; PCV, Phenotypic coefficients of variations; h^2, Heritability; GA, Genetic advance; CV, Coefficient of variability; SE, Standard error; GAPM, Genetic advance as % of mean.

Table 2 Correlation and path analysis between yield and quality traits in triticale

Characters	GPC	TW	GAS	PRS	SDS-SV	TGW	GY
Character association							
GPC	1.00	0.51*	0.66**	-0.67**	0.50*	0.56**	0.61**
TW		1.00	0.28	-0.31	0.85**	0.37	0.44*
GAS			1.00	-0.68**	0.47*	0.24	0.29
PRS				1.00	-0.70**	-0.65**	-0.68**
SDS-SV					1.00	0.46*	0.68**
TGW						1.00	0.67**
Path matrix of traits							
GPC	0.06	0.03	0.03	-0.13	0.01	0.07	0.56
TW	0.06	0.13	0.07	0.05	0.23	0.06	0.37
GAS	-0.19	0.04	-0.07	0.20	-0.09	-0.07	0.26
PRS	0.63	0.25	0.49	-0.70	0.59	0.38	0.71
SDS-SV	0.03	0.02	0.11	0.09	0.04	0.16	0.63
TGW	0.20	0.05	0.10	-0.12	0.15	0.30	0.62
Partial R^2	0.02	0.04	0.05	0.43	0.04	0.18	

*, ** significant at 5% and 1%; R^2, 0.653; Residual effect, 0.578. GPC, Grain protein content; TW, Test weight; GAS, Grain appearance score; PRS, Phenol reaction score; SDS-SV, Sedimentation value; TGW, 1000-grains weight; GY, Grain yield/plot.

and IV (T3949, T3973, T3974 and T3975) consisting of four genotypes each. These were followed by cluster V containing two genotypes (T3978 and TL2908) whereas cluster I was the smallest possessing single genotype T3868. The intra-cluster distances ranged from 0 (cluster I) to 2.971 (cluster V). Cluster I and IV (15.084) showed maximum inter-cluster distance followed by clusters I and III (12.593) and clusters IV and V (10.772) whereas clusters II and V (3.577) showed minimum inter-cluster distance. The presence of diversity among the clusters was shown by greater range and magnitude of inter-cluster distance than intra-cluster distances. The use of germplasm lines in hybridisation from these distant clusters are likely to produce more heterotic hybrids vis-a-vis transgressive segregants. The cluster mean values indicated highest values for GPC (13.02%), SDS-SV (38.34), TGW (41.0 g) and GY (4.87 kg) in cluster IV whereas high mean values for TW (72.78 kg/hl) and GAS (5.09) were shown in cluster III. The cluster mean values indicated accommodation of best performing genotypes in cluster IV for most of the traits except TW and GAS. As lower values for PRS is desirable, lowest cluster mean for this trait (1.68) was also observed in cluster IV. The proximity index indicated minimum Euclidean distance (0.027) between T3949 and T3974 whereas T3868 and T3977 were found highly distant (0.482) which may be utilised in hybridisation programme to generate maximum variability. In addition, the genotypes T3940, T3949, T3973, T3974, T3975, T3979 were identified as promising on the basis of genetic divergence and per se performance for several traits particularly for test weight, 1000-grain weight and grain appearance score and can be used in future breeding programme for improving grain yield and component traits in order to enhance the productivity of Triticale.

Principal component analysis (PCA): The PCA indicated contribution of seven factors for variability of which first two factors F_1 (54.9%) and F_2 (15.9%) contributed maximum upto 70.77% variability. The contribution of variables in PCA revealed maximum contribution of PRS (21.3%) followed by SDS-SV (18.7%), GPC (17.7%) and GY (15.1%) in total variability shown due to PC_1 whereas TW (57.9%) and SDS-SV (16.0%) contributed maximum in PC2. The biplot analysis (Fig. 2) indicated clustering pattern of genotypes as well as association of various traits. Traits namely GPC, TGW and GAS constitute first cluster alongwith GY whereas another cluster was formed by SDS-SV and TW. The distance from the centroid indicates the contribution of traits towards variability which was found maximum by TW (0.761) in PC2. Negative associations between all the

Table 3 Clustering pattern and Intra and inter-cluster distances in triticale

Cluster	Entries	Genotypes	Intra and inter-cluster distances				
			I	II	III	IV	V
I	01	T3868	0	6.159	12.593	15.084	7.507
II	09	T3895, T3901, T3902, T3937, T3938, T3979, T3984, TL2942, TL3969		1.745	6.714	9.333	3.577
III	04	T3940, T3956, T3977, T3983			2.552	6.819	6.108
IV	04	T3949, T3973, T3974, T3975				1.570	10.772
V	02	T3978, TL2908					2.971

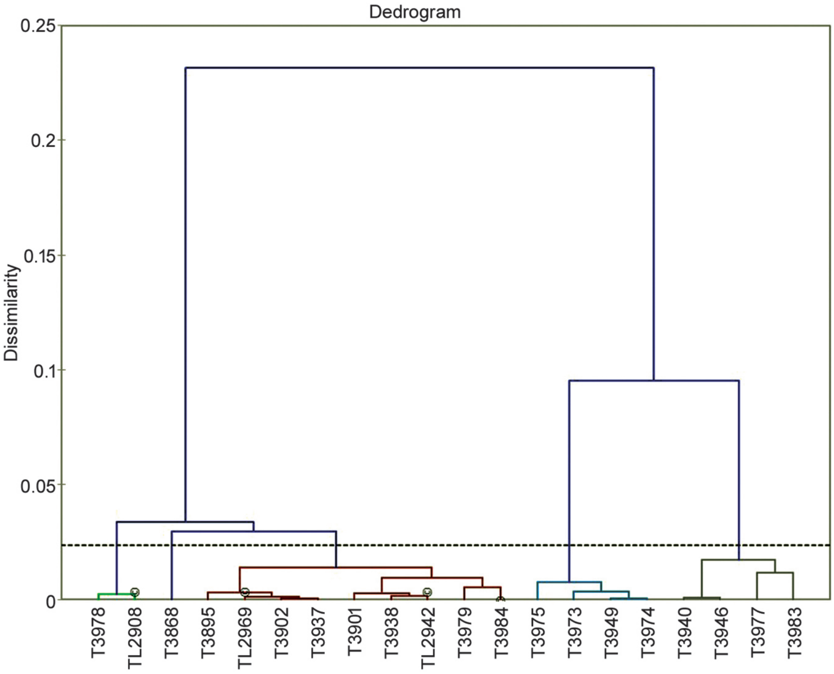

Fig. 1 Dendrogram showing diversity and clustering among triticale genotypes.

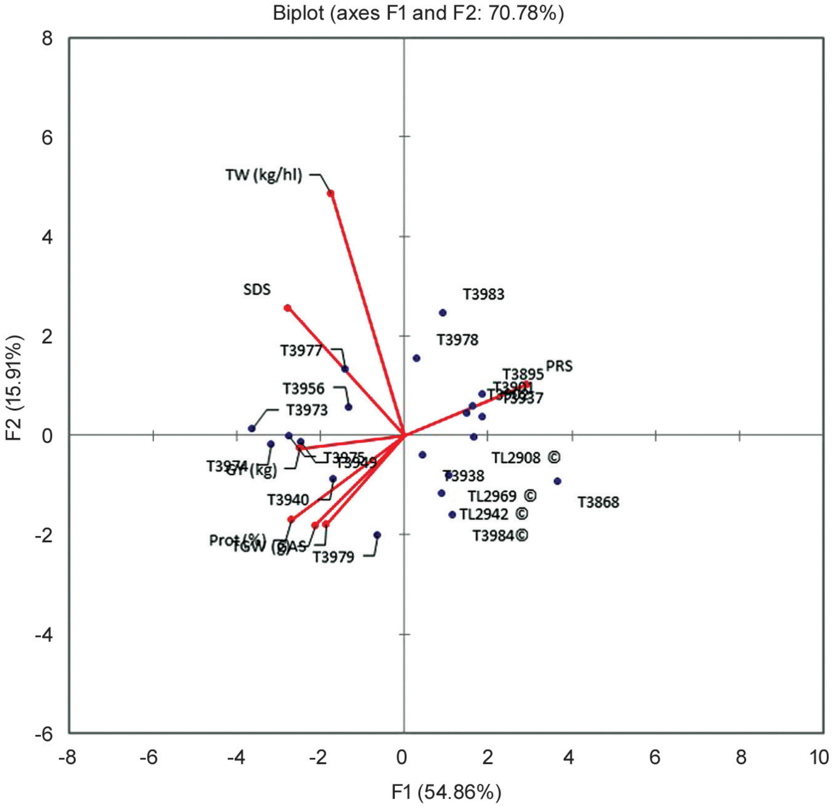

Fig. 2 PCA biplot of the seven traits among 20 triticale genotypes.

traits and factors F_1 and F_2 except PRS (0.905) in F_1 and TW (0.803), SDS-SV (0.423) and PRS (0.169) in F_2 were observed. The biplot analysis indicated clustering pattern of genotypes as SDS-SV well as association of various traits where length of arrows indicated the relative size of contribution of the trait in the PCA. The associations and diversity pattern reflected in PCA are found in accordance to character association and genetic divergence studies similar to reported by Kumar *et al.* (2016).

In conclusion, this study has clearly indicated the need for of emphasising test weight, grain appearance score and 1000-grain weight for improving grain yield in Triticale. These variables have maximum potential of selection for grain yield improvement as these traits possessed significantly positive correlation coupled with high direct effect on yield. Genotypes T3974, T3973, T3949 and T3975 were found to be promising genotypes for multiple traits which can be used as donor parent for yield and quality improvement in Triticale.

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