# ASSESSMENT OF GENETIC DIVERSITY AND IDENTIFICATION OF SUPERIOR DONORS FOR HIGHER YIELD, NUTRITIONAL AND PROCESSING TRAITS IN TOMATO THROUGH HIERARCHICAL CLUSTERING ANALYSIS

Comment [A1]: Add scientific name of the crop


**Abstract**

The present investigation was carried out in the Department of Horticulture, BAC, BAU, Sabour, Bihar, with 22 diverse genotypes of tomato germplasms collected from diverse locations and sources. The 22 genotypes were assessed for their phonological, morphological, yield, nutritional and processing quality attributes. The hierarchical clustering analysis based on the Euclidean distance and Ward's method were applied on these genotypes separately based on two segments namely the morphological and yield attributes; and the nutritional and processing quality attributes, aimed to find superior genotypes from both segments. Further, through the same analysis, the genetic diversity among the 22 genotypes for these traits were also established. Additionally, the association among the plant traits are also partly reported with a heatmap visualization. Our findings suggest that the genotypes 2019/TODVAR-4, 2019/TODVAR-5, 2019/TODVAR-2, 2019/TODVAR-7, and 2019/TOLOCVRES-4 exhibit superior processing quality attributes. In particular, genotypes with higher yields coupled with favorable nutritional and processing qualities significantly enhance the success of breeding programs. Notably, genotype 2019/TODVAR-4 has been identified as particularly suitable for developing cultivars with excellent processing qualities.



**Keywords:** Tomato; Genetic diversity; Nutritional& processing quality; Identification of superior genotypes: Hierarchical clustering.


Comment [A2]: Expand and write AND instead of &

## 1. Introduction

Tomato(*Solanumlycopersicum*L.)isoneofthemostimportantwarmseasonfruitvegetable cropsgrownthroughouttheworld.Indiaholdssecondpositionasaleadingproduceroftomatointhe world.
ItbelongstothefamilySolanaceaeandhaschromosomenumber2n=24.Itproduceschasmogamo usflowerswherepredominatelyself-pollinationisseen.
Itismostlyknownas“Protectivefood”duetoitsnutritivevalue,antioxidantmoleculessuchascarot enoids,particularly
lycopene,ascorbicacid,vitaminEandphenolcompounds,mainlyflavonoids.It alsocontainsminerals likeiron,phosphorus, and potassium (Brar *et al.,* 1998). Tomatofruitsarecanbeusedbothasfreshaswellasprocessedform.
Itranksfirstinthelistofprocessingvegetables inIndia.Fruits usedformakingprocessedfoodsincludeketchup,puree,powder,paste,sauces,andsoups.
Lycopenehasbeenshowninstudiestooffersignificanthealthbenefits,sinceitprotectsagainsttheo xidationoffreeradicals.Itmayalsostoplow-
densitylipoproteinsfromoxidising(badcholesterol).Becauselycopeneisapowerfulantioxidant.
Ithasbeenassociatedtoadecreasedriskofsomeformsofcancerinthosewhoconsumeitbecauseofit snutritionalcontent.Humanbeings(Chernet,
2013).Itprotectsthehumanbodyagainstdiseaseduetoitsnutritiousvalue. A typical processing genotype should possess high TSS (>5$^{o}$ Brix), minimum sugar acid ratio (15:1), lycopene (>10mg/100g FW) with a high colour value of (>2) and low pH (<4.3) that improve quality of the valued-added products, and reduce energy consumption and cost of processing (NAAS, 2020).

Bychoosingapexgenotypesforquickincreaseinyieldandotherhorticulturaltraits,geneticv ariabilityplaysasignificantroleincropbreedingprogrammes.Themorethegeneticdiversityinqu alitativeandquantitativefeatures,thehighertheprospectsofcropdevelopmentthroughselection.
Themultivariateanalysisusinghierarchical clustering analysis using Ward’s algorithmisavaluabletooltoquantifythedegreeofdivergenceatgeneticlevel.Whileformulati ngthetomatocropimprovementprogram,understandingaboutthenatureanddegreeofgeneti cdivergenceavailableinthegermplasmplaysapivotalrole.Itiswellrecognizedthattheuseofdi verseparentsresultsinsuperiorhybridsanddesirablerecombinants.Thus,geneticdivergence existinginthepopulationhelpsinselectingsuitableparentsforhybridizationprogram.

Considering,thepotentialanddemandoftomatocrop,thereisanurgentneedtoidentifyandd evelopvarieties/genotypessuitableforcultivationunderdifferentagro-
climaticconditionsofSabour,Bhagalpur,
Bihar.However,alotofworkhasbeencarriedoutoncropimprovementintomatoelsewhere,butme agerworkinthislinehasbeencarriedoutforBhagalpurregion.Keepingtheabovethemesinview,th is investigation was undertaken to assess the genetic diversity among the evaluated genotypes and to identify superior and distant lines to deploy them in the future breeding programs for processing and nutritional quality.

## 2. Materials and methods

ThepresentexperimentconductedatDepartment
ofHorticulture,BAC,BAU,Sabour,Bhagalpurduring*rabi*seasonoftheyear2021-
2022.Geographically,Sabourissituatedat25°15'40”Nlatitudeand87°2'42”Elongitudewithanalti tudeof45.57meterabovemeansealevel(MSL).Thisplaceischaracterizedbysemi- aridandsub-
tropical climatewith drysummer,average precipitation andcoldwinter. The experimental

materials included 22 diverse genotypes of tomato collected from diverse locations and sources (Table 1).

Comment [A3]: No check varieties in the study?

Table 1. List of tomato genotypes and their sources

| S. No. | Name ofVariety | Sources |
|---|---|---|
| 1 | 2019/TODVAR-1 | AICRP on vegetablecrops |
| 2 | 2019/TODVAR-2 | AICRP on vegetablecrops |
| 3 | 2019/TODVAR-3 | AICRP on vegetablecrops |
| 4 | 2019/TODVAR-4 | AICRP on vegetablecrops |
| 5 | 2019/TODVAR-5 | AICRP on vegetablecrops |
| 6 | 2019/TODVAR-6 | AICRP on vegetablecrops |
| 7 | 2019/TODVAR-7 | AICRP on vegetablecrops |
| 8 | 2019/TODVAR-9 | AICRP on vegetablecrops |
| 9 | 2019//TOLCVRES-2 | AICRP on vegetablecrops |
| 10 | 2019//TOLCVRES-3 | AICRP on vegetablecrops |
| 11 | 2019//TOLCVRES-4 | AICRP on vegetablecrops |
| 12 | 2019//TOLCVRES-5 | AICRP on vegetablecrops |
| 13 | 2019//TOLCVRES-6 | AICRP on vegetablecrops |
| 14 | 2019//TOLCVRES-7 | AICRP on vegetablecrops |
| 15 | 2019//TOLCVRES-8 | AICRP on vegetablecrops |
| 16 | PunjabChhuhara | PAU,Ludhiana, Punjab |
| 17 | KashiAmrit | IIVR, Varanasi, Uttar Pradesh |
| 18 | KashiChayan | IIVR, Varanasi, Uttar Pradesh |
| 19 | IIHR-2614 | IIHR,Bengaluru, Karnataka |
| 20 | Arka Vikas | IIHR,Bengaluru, Karnataka |
| 21 | Arka Alok | IIHR,Bengaluru, Karnataka |
| 22 | BRDT-1 | BAU,Sabour, Bihar |

Comment [A4]: Are these varieties or genotypes? Specify



The crop production was carried out by following the standard package of practices recommended for Sabour region of Bihar. The plant morphological traits namely days to 50% flowering (DFF), primary branches plant$^{-1}$ (PBP), plant height (PH), days to first fruit set (DFFS), days to maturity (DM), average single fruit weight (FW), locules fruit$^{-1}$ (LF), fruit polar diameter (FPD), fruit equatorial diameter (FED), pericarp thickness (PT), fruits plant$^{-1}$ (FP), fruit yield plant$^{-1}$ (FYP) were observed in randomly selected four plants. For the same plants, biochemical attributes namely the total lycopene content (LYC), titrable acidity (TA), total soluble solids (TSS), ascorbic acid (ASC), total carotenoids (CAR) and β-carotene (B-CAR) were determined. Thetotallycopenecontentoftomatofruitwasdeterminedusing Lee's (2001) methodwithsome

modifications. Titrableacidity was determined by using titration method (AOAC, 2000). The TSS was assessed with a digital refractometer. The amount of ascorbic acid in the juice was measured by titrating it against 2,6-dichlorophenolindophenol sdye (AOAC, 2000). Total carotenoids and β-carotene contents were estimated as per Sadasivam and Manickam (1996) using composite sample of five fruits from each replication, in a UV-VIS Spectrophotometer (Labman) at 452 nm wavelength. The analysis of variance (ANOVA) of the observations recorded on different attributes was performed according to Panse and Sukhatme's standard approach (1985). The significance of the results was determined using the F table values (Fisher and Yates, 1963). Grouping of genotypes and subsequent heatmap for chromic visualization of mean performances of the genotypes were done based on plant morphological, yield, nutritional and processing quality attributes using the cluster analysis through Ward's method and Euclidian distance in RStudio (Version 4.3.3, RStudio Team, 2020).

## 3. Results and discussion

### 3.1. Genetic diversity among 22 tomato genotypes based on the morphological and yield attributes

The morphological and yield attributes namely the days to 50% flowering (DFF), days to first fruit set (DFFS), days to fruit maturity (DFM), number of fruits plant$^{-1}$ (NFP), number of primary braches plant$^{-1}$ (NPB), plant height at 90 days after sowing (PH90) fruit yield plant$^{-1}$ (FYP) were recorded from the plants of 22 tomato genotypes. The hierarchical clustering pattern, presented in Fig. 1a, was constructed based on Euclidean distance among the genotypes using the Ward's algorithm. The clustering analysis grouped 22 gtenotypes into 5 clusters: cluster I with 8 genotypes (2019/TODVAR-2, Punjab chhuhara, 2019/TODVAR-5, 2019/TOLCVRES-7, 2019/TODVAR-6, Kashi Amrit, 2019/TOLCVRES-2 and 2019/TOLCVRES-6); cluster II with 2 genotypes (IIHR-2614 and Arka Vikas); cluster III with 3 genotypes (2019/TODVAR-1, Kashi Chayan (C) and 2019/TODVAR-3); cluster IV with 2 genotypes (Arka Alok and BRDT-1) and cluster V with 7 genotypes (2019/TOLCVRES-5, 2019/TODVAR-7, 2019/TODVAR-4, 2019/TOLCVRES-8, 2019/TOLCVRES-4, 2019/TODVAR-9 and 2019/TOLCVRES-3). From this, it is evident that the evaluated genotypes are diverse and thus suitable for subjecting them for breeding cultivars with high yielding traits. Nankar et al. (2020) studied the diversity of 150 accessions of tomato genotypes across the world for their morphological and biochemical traits, through hierarchical clustering method. Their findings revealed better genotypes for future breeding programs in tomato. Similar studies were also performed by Mahesha *et al.* (2006), Meena and Bahadur (2015), Jogi *et al.* (2008), Rana and Singh (2010), and Lekshmi and Celine (2016).

**Comment [A6]:** Check the spelling of genotypes

**Comment [A7]:** Check the reference writing format and change accordingly in the whole manuscript

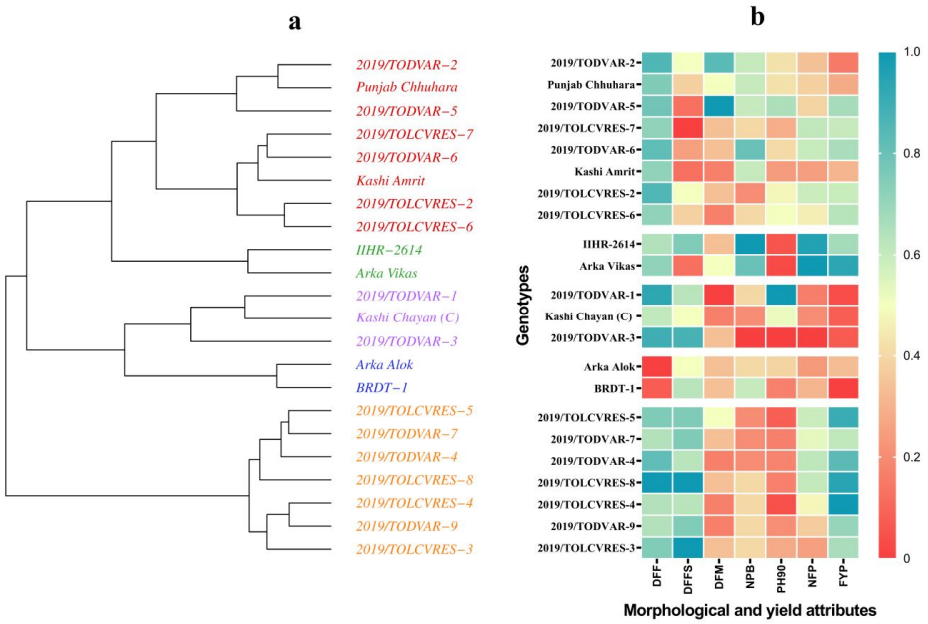


Fig. 1. Genetic diversity based on morphological and yield attributes. a) dendrogram from Ward's algorithm; b) heatmap showing the performance of the 22 genotypes

### 3.2. Genetic diversity among 22 tomato genotypes based on the nutritional and quality attributes of fruits

The harvested fruits of the 22 genotypes were assessed for their nutritional attributes namely lycopene, titrable acidity, total soluble solids (TSS), ascorbic acid (ASC), total carotenoids (CAR) and β-carotene (B-CAR) and processing quality attributes like fruit weight (FW), number of locules fruit $^{-1}$ (NLF), fruit polar diameter (FPD), fruit equatorial diameter (FED) and pericarp thickness (PT). The Fig. 2a shows the clustering pattern based on these traits, the 22 genotypes were catagorized into 5 clusters: cluster I with 3 genotypes (Kashi Amrit, Punjab chhuhara, 2019/TOLCVRES-2); cluster II with 3 genotypes (2019/TODVAR-5, 2019/TOLCVRES-4, 2019/TOLCVRES-3); cluster III with 6 genotypes (2019/TODVAR-9, 2019/TOLCVRES-8, 2019/TOLCVRES-5, IIHR-2614, 2019/TODVAR-4 and Arka Vikas); cluster IV with 7 genotypes (Kashi Chayan (C), Arka Alok, 2019/TODVAR-2, 2019/TODVAR-7, BRDT-1, 2019/TODVAR-3 and 2019/TOLCVRES-7); cluster V with 3 genotypes (2019/TODVAR-1, 2019/TOLCVRES-6 and 2019/TODVAR-6). This shows that the evaluated genotypes are diverse for the nutritional and processing traits too, indicating their prominent potential for utilization as donors in tomato breeding programs aimed at enhanced nutritional and processing quality attributes.Lekshmi and Celine (2016) reported that wide ranges of variation were observed among the characters studied which have a great interest for polyhouse tomato breeding. Genetic divergence analysis was carried out using MahalanobisD2 statistics and the 40 tomato genotypes were grouped into eight clusters. Cluster I was the largest cluster with twenty four genotypes followed by cluster II with ten genotypes and all other clusters were solitary. The highest intra- cluster distance

**Comment [A8]:** Is it titrable or titratable? Check and correct the spelling in the complete manuscript

**Comment [A9]:** $D^2$not D2

**Comment [A10]:** 22 or 40 genotypes?

**Comment [A11]:** Number of genotypes how varied in the study?

was noticed in cluster II followed by cluster I. The highest inter- cluster distance was observed between clusters VII and VIII, followed by clusters IV and VIII. Lycopene content and truss per plant had maximum contribution towards total divergence followed by fruit length, fruit weight and yield per plant.

Comment [A12]: What is the meaning of truss?

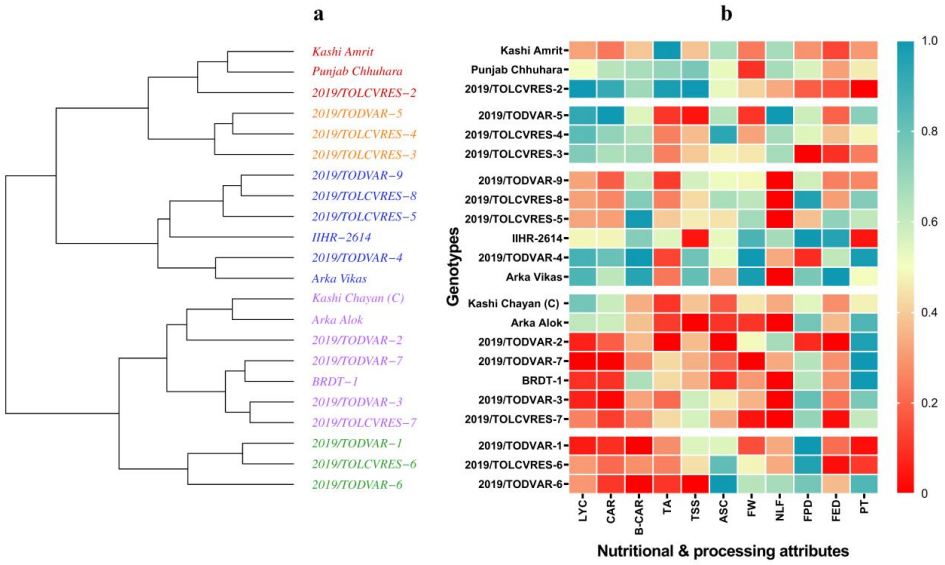


Fig. 2. Genetic diversity based on nutritional and processing quality attributes. a) dendrogram from Ward's algorithm; b) heatmap showing the performance of the 22 genotypes

### 3.3.Identification of superior donors for breeding towards higher yield, nutritional and processing quality attributes

Fig. 1b shows the mean performance trend of the morphological and yield attributes of 22 genotypes (data not shown). A correlation among the morphological traits were hard found, except for das to 50% flowering and days to first fruit set and yield with fruit related traits. The clusters 2 and 5 had higher yielding genotypes, whereas the genotypes under cluster 3 and 4 had poor performers, while the genotypes under cluster1 were intermediate to both high and low extremes. The heatmap also showed a negative correlation between fruits yield plant$^{-1}$ and plant height across all the genotypes. We found it interesting that the poor yielders of cluster 3 and 4 as well as the good yielders of cluster 5 showed lower number of primary branches and number of fruits plant$^{-1}$. Whereas the genotypes under cluster 2 (IIHR-2614 and Arka Vikas) showed higher yield coupled with higher number of branches plant$^{-1}$ and number of fruits plant$^{-1}$. More are the primary branches, more is the number of fruits plant$^{-1}$, hence is the yield. Thus, the number of primary branches plant$^{-1}$ is a primary and direct selection trait towards improving yield. The genotypes Arka Vikas, 2019/TOLCVRES-4, 2019/TOLCVRES-8, 2019/TOLCVRES-5 and 2019/TOLCVRES-4 are identified as the higher yielders and therefore can be used in breeding programs for yield maximization, owing to their exceptional performance surpassing the check cv. Kashi Chayan. In addition to the identification of high yielding lines, our results also shed insights on the scopes for improving othr genotypes. The line IIFR-2614 showed the highest number of primary

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braches plant$^{-1}$, however it couldn't be a superior yielder and was found shorter in yield than the highest yielders identified and mentioned above. Since, number of primary branches plant$^{-1}$ is a major target for yield maximization, IIHR-2614 could be used in hybridization efforts with the high yielders of cluster 3 and 5. The increase in number of primary branches plant$^{-1}$ in these poor branch-bearing genotypes would significantly boost the yield by manifolds. Essentially, the underlying cause for lower yields of IIHR-2614 despite its higher number of primary branches plant$^{-1}$ needs further investigation to identify the negative drivers of the yield reduction. The genotypes under cluster 4 (Arka Alok and BRDT-1) showed earliest flowering and maturity but are poor yielders. However, early maturity of these genotypes could be incorporated into high yielders of clusters 3 and 5, thereby coupling early maturity and higher yielders, enabling farmers to find places and profits in lean seasons. In addition to the days to 50% flowering, the days to first fruit set and days to fruit maturity are also important traits deciding the early harvest. Though Arka Alok and BRDT-1 showed the earliest flowering, the earliest fruit set and maturity were seen with 2019/TOLCVRES-7 (cluster 1) and 2019/TODVAR-1 (cluster 3). These lines could also be deployed to breed early and high yielding cultivars. Since the fruit yield and its drivers showed no significant correlation (data not shown), breeding superior lines with higher yield and early maturity, with suitableplant architecture (plant height and number branches plant$^{-1}$) is possible through hybridization.

Fig. 2b gives a comprehensive picture on the phenotypic variability, genotypic diversity, correlation and lines that scored maximum for nutritional and processing qualities. There was hardly a correlation among the nutritional traits and processing quality traits, except for positive correlation among the total carotenoids, β-carotene and lycopene; fruit weight and β-carotene; fruit equatorial diameter and β-carotene (data not shown). The desirable fruit weight for processing is >80g. In our investigation, only the genotypes 2019/TODVAR-4 had an average fruit weight of 80.75 g (data not shown). The desirable number of locules fruit$^{-1}$ is 2-4, which was observed in all the genotypes, except in 2019/TODVAR-5. The pericarp thickness greater than 0.4cm is desirable for processing cultivars. Most of the genotypes under the study showed a pericarp thickness > 0.4cm. Particularly, 2019/TODVAR-7 and BRDT-1 showed the highest pericarp thickness of 7.42 mm, followed by 2019/TODVAR-4 (7.33 mm) and 2019/TODVAR-2 (7.26 mm). The desirable lycopene in fruit for processing purpose is >8.5 mg 100g$^{-1}$. The highest lycopene contents were seen with 2019/TOCVRES-2 (10.98), followed by 2019/TODVAR-5 (10.45) and 2019/TODVAR-4 (10.15). The titrableacididty needs to be as low as 0.40%. The genotypes 2019/TODVAR-2 showed the least acidity (0.18%), followed by 2019/TODVAR-5, 2019/TODVAR-6 and Kashi Chayan (C) (0.25%). The desirable TSS content is > 5.5$^{o}$ Brix. The ascorbic acid content for processing types is recommended to be >25 mg 100mg$^{-1}$. In our investigation, none of the genotypes had an ascorbic acid content > 25 mg 100mg$^{-1}$. However, 2019/TODVAR-6 (24.28) and 2019/TOCVRES-4 (23.27) and 2019/TOCVRES-6 (21.44) showed greater ascorbic acid contents.

**Comment [A15]:** Check the spelling of acidity also

Our findings reveal that the genotypes namely 2019/TODVAR-4, 2019/TODVAR-5, 2019/TODVAR-2, 2019/TODVAR-7 AND 2019/TOLOCVRES-4 are superior in the processing quality attributes. However, the genotypes with higher yield and good nutritional and processing qualities make the breeding efforts more fruitful. In this context, the

genotype 2019/TODVAR-4 is also found to be suitable for developing cultivars with good processing qualities. It is important to note that these genotypes are better performers, both by means of yield; and nutritional and processing attributes, thereby are potential donors in future breeding programs. These genotypes would form a population upon which new cultivars could be develop, that would possibly surpass the existing cultivars for processing namely Punjab Chhuhara and Kashi Chayan (C). Further efforts in this direction with these genotypes would fulfil the never-ending and ever-increasing demand for food and nutritional security of the human kind.

Comment [A16]: There by .....give space

## 4. Conclusion

Based on the morphological and yield attributes, the clustering analysis grouped 22 gtenotypes into 5 clusters: cluster I with 8 genotypes (2019/TODVAR-2, Punjab chhuhara, 2019/TODVAR-5, 2019/TOLCVRES-7, 2019/TODVAR-6, Kashi Amrit, 2019/TOLCVRES-2 and 2019/TOLCVRES-6); cluster II with 2 genotypes (IIHR-2614 and Arka Vikas); cluster III with 3 genotypes (2019/TODVAR-1, Kashi Chayan (C) and 2019/TODVAR-3); cluster IV with 2 genotypes (Arka Alok and BRDT-1) and cluster V with 7 genotypes (2019/TOLCVRES-5, 2019/TODVAR-7, 2019/TODVAR-4, 2019/TOLCVRES-8, 2019/TOLCVRES-4, 2019/TODVAR-9 and 2019/TOLCVRES-3). Similarly, the clustering pattern based on the nutritional and processing traits, the 22 genotypes were catagorized into 5 clusters: cluster I with 3 genotypes (Kashi Amrit, Punjab chhuhara, 2019/TOLCVRES-2); cluster II with 3 genotypes (2019/TODVAR-5, 2019/TOLCVRES-4, 2019/TOLCVRES-3); cluster III with 6 genotypes (2019/TODVAR-9, 2019/TOLCVRES-8, 2019/TOLCVRES-5, IIHR-2614, 2019/TODVAR-4 and Arka Vikas); cluster IV with 7 genotypes (Kashi Chayan (C), Arka Alok, 2019/TODVAR-2, 2019/TODVAR-7, BRDT-1, 2019/TODVAR-3 and 2019/TOLCVRES-7); cluster V with 3 genotypes (2019/TODVAR-1, 2019/TOLCVRES-6 and 2019/TODVAR-6). This shows that the evaluated genotypes are diverse for their morphological, nutritional and processing traits along with yield levels, indicating their prominent potential for utilization as donors in tomato breeding programs aimed at enhanced nutritional and processing quality attributes coupled with high yields. Our results recommend that genotypes namely 2019/TODVAR-4, 2019/TODVAR-5, 2019/TODVAR-2, 2019/TODVAR-7 AND 2019/TOLOCVRES-4 are superior in the processing quality attributes. Particularly, the genotypes with higher yield and good nutritional and processing qualities make the breeding efforts more fruitful. In this context, the genotype 2019/TODVAR-4 is also found to be suitable for developing cultivars with good processing qualities.



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Comment [A17]: Scientific name should be italicised in all the refernces

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