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# SCREENING OF THIRD FILIAL (F3) SEGREGATING POPULATION FOR SALT TOLERANCE IN CEREAL: A REVIEW

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**Abstract:** Cereal crops such as maize, wheat, rice, and others are cultivated in every part of the world. However, cereals crop cultivation globally has been affected by salinity stress. Salinity stress causes a reduction in the growth, yield, and productivity of cereal crops. Hence, to overcome the problem related to salinity stress, several plans are made to develop a salinity tolerance cereal variety. Therefore, various strategies, from phenotypic and molecular screening, have been introduced to develop salinity tolerance cereal varieties. Salinity tolerance is a crucial trait that must be inserted into cereal crops to maximize the yield productivity of cereals crops. The objective of this review is to undergo screening for salinity tolerance in the third filial (F3) segregating population of cereals to identify the large amounts of lines correlated with salt tolerance which were further used in the breeding process. Besides, the selection process of F3 and other populations of the cereals is conducted on yield and yield components and the correlation between traits linked with salt tolerance. Thus, this review study will focus on the screening and selection process of the F3 and other generations on salinity-tolerant and high-yielding cereals developments.

Keywords: Screening, doubled haploid, cereals, F3 population, salt tolerance.

## Introduction

Salinity is one of the main problems that are affecting the production of agriculture around the globe. Based on the current situation, it is estimated that about 45 million hectares of irrigated lands, equivalent to 19.5%, are highly affected by salinity (Wild, 2003). The presence of salinity in the land area is devastating to the crops as it can disturb plant growth and development in all life stages. Besides, the accumulation of salt in a very high amount can significantly decline the yield per capita. Cereal crops like rice, wheat, and barley are affected by salinity in the irrigated land area. As reported by Liu *et al*. (2019), increasing soil salinization is one of the major challenges to rice production, millions of hectares of land in the humid regions of South and Southeast Asia where rice is the main food crop are uncultivable, or rice is grown with very low agronomical yields.

Hence, to ensure that the cereal crops can sustain under the presence of high salinity and to respond to the salinity stress, cereal crops develop a salinity tolerance mechanism spectrum via modifications of morphological, physiological, and metabolic processes. Khan *et al*. (2016) stated that cereal crops undergo different salt tolerance mechanisms to overcome salinity and develop salinity tolerance characteristics at different life stages. However, despite the presence of the salinity tolerance mechanism in crops, some problems exist, such as the difficulty in understanding the complexity of the salinity tolerance mechanism and also the crops' tolerance level to the salinity stress depends on the morphological, physiological, biochemical, and molecular components or changes in traits (Kakar *et al*., 2019). Generally, the effect of salt stress on the plant is evident in terms of agronomical (morphological), physiological, and biochemical. All these effects result from the interaction between the salt molecules and the plant cells. High salinity in soil gravely affects the germination of seeds and, in extreme conditions, leads to dead seeds through exosmosis. Salt stress seriously limits growth and grain yield in rice (Liu *et al*., 2019). Plant cells store excess salt in the vacuole or sequestrate them into older plant organs or dead cells to maintain the osmotic condition. Electron transport chains in mitochondria can overflow, deregulate, or become disrupted by salinity stress leading to the accumulation of toxic compounds (Mwando *et al*., 2020).

Souleymane *et al.* (2015) state that understanding the genetic control of plant traits exposed to salinity stress is crucial in breeding salt-tolerant rice cultivars. The second filial (F2) to the sixth filial (F6) generations are essential stages for the selection and evaluation of the segregants during breeding protocols (Ahmad *et al*., 2018). Brown and Caligari (2008) revealed that unpredictable environmental factors can affect the highest-yielding progeny lines derived from F2 and F3 generations and may lead to inferior segregants. Molla *et al*. (2015) expressed that approximately 200 genes have been identified in salt stress at the seedling stage using mutants and gene overexpression and knockout approaches. The experiment of Liu *et al*. (2019) showed that salt-tolerant rice seedlings might not necessarily yield high when grown in saline conditions, implying the disparity in the genetic basis controlling between the two different growth stages.

Therefore, to solve the salinity problems and to avoid the salinity stress causing more damage to the cereal crops, scientists have introduced several strategies in plant breeding that are useful and highly significant to develop a cereal crop with salt tolerance characteristics. Salinity tolerance is a polygenic trait (Islam *et al*., 2010). One of the strategies that are employed is the screening process which is divided into two methods which, are phenotypic and molecular screening. Phenotypic screening involves identifying traits correlated to salinity tolerance via specific salt tolerance criteria, while molecular screening applies Quantitative Traits Loci (QTL) mapping to identify QTLs related to salt tolerance via molecular markers (Yen & Lin, 2011). Besides, selection response for yield and yield component characters and a correlation between traits leading to salinity tolerance in cereals is another strategy that scientists introduce. This review was conducted with a third filial (F3) segregating population as the major aim to be employed in the screening process in finding the salt tolerance germplasm and the selection of yield and yield component characteristics and correlation between traits related to salt tolerance in cereal.

## Methods

### *Data Collection*

This review study focuses on the screening process of the F3 segregating population of cereals for salt tolerance, the selection process of the F3 population according to yield and yield component characteristics, and a correlation between salinity tolerance traits. Hence, to gather the information based on the past study on the screening and selection process of F3 cereals, many literature sources were used in this review study, such as postgraduate thesis, article journal, past research papers, textbooks, and manuscripts. Most of the literature sources are from the internet, where several trustful websites are used in the literature or information searching, such as ScienceDirect, ResearchGate, Springer, MDPI, Frontier in Plant Science, Semantic Scholar, and others.

The F3 segregating population and other populations were mainly targeted for collecting data or information. The data or information that is collected and gathered from various journals and publications are mainly related to the selection of yield and yield component, the correlation between salt tolerance traits, breeding methods, phenotypic screening, molecular screening via QTLs mapping using molecular markers such as Simple Sequence Repeat (SSR), Inter Simple Sequence Repeat (ISSR), and Random Amplified Polymorphic DNA (RAPD), and also Genome-Wide Association Study (GWAS) for salt tolerance in cereals.

## Discussion

### *Selection Based on Yield and Yield Components*

Direct yield estimations are not significant in the early plant breeding phases since the selection process is harder and more complicated to achieve. Hence, to estimate selection gain and yield intensity, the strategies that need to be conducted bypass the genetic potential from one segregating generation to the next for a specific characteristic (Jayaprakash *et al*., 2017). Augustina *et al*. (2013) stated that yield components or attributes that strongly link with yield must be well understood since they may be used to drive selection criteria or indices to enhance the productivity in a new cereal crop variety.

The most important thing to be achieved is to select yield components that cause the yield increased significantly. Highly heritable and genetically independent or related features improve grain productivity. Surek and Beser (2005) suggested the selection for yield and yield components criteria are the grain weight, the number of grains per panicle, or several panicles per unit area. Table 1 summarizes the finding on the selection response on yield and yield component characters in cereals. Various previous study shows that the selection of F3 generation based on yield and yield components are advantageous due to the positive correlation between the selection also attributes to the maximum performance of yield and yield components found in the F3 populations.

Table 1: Summary of selection response on yield and yield component characters in cereals

| Research | Yield and Yield Component Characters | Cereals | References |
|---|---|---|---|
| Components of yield & yield of grain selection in the early generation of temperate rice | Correlation between the number of grains per panicle and grain yield in the F3 population is significant & positive | Rice | Surek & Beser (2003) |
| Yield and yield components selections in white oat | High average weight and yield of grain for F3 & F4 populations | Oat | Benin *et al.* (2005) |
| Selection response in F3 wheat populations for grain yield | Maximum spike length of spike & grain spikes number in the F3 population | Wheat | Aziz *et al.* (2018) |
| Selection for grain yield in rabi sorghum F3 generations | F3 population has high relative efficiency and genetic advance on primaries and yield per plant, panicle, and 100-grain weight | Sorghum | Prasad and Biradar (2019) |
| Selection response for the yield of grain and yield attributing traits on F3 and F4 generations in aromatic rice | Number of panicles per plant and days to 50% flowering has a positive value correlation | Rice | Koli and Patidar (2018) |
| Selection for F3 white maize population for yield potential | F3 lines have the highest performance of grain yield per plant | Maize | Nessa Shompa *et al.* (2020) |
| Studies on the selection of bread wheat F3 population for grain yield | F3 crosses show a higher amount of grain yield with medium height compared to the parents | Wheat | Darwish *et al.* (2018) |
| Yield and yield component characters performance of F2 and F3 generation of rice | The positive correlation is shown in the F3 population for grain number per panicle, the height of the plant, length of panicle, and flowering | Rice | Suwarto and Susanto (2015) |

### *Correlation between Traits for Salinity Tolerance*

The correlation between traits for salinity tolerance is crucial in the selection process in the F3 segregating population as it indicates the indirect selection probability to achieve success. In the early selection process of cereal crops, the study on the correlation between salt tolerance traits mostly applies the correlation coefficient with more than one trait or attribute is sought. The knowledge of the correlation between traits helps plant breeders in terms of understanding the components of yield.

Al-Ashkar *et al*. (2021) reported that the knowledge about the correlation between traits in the salinity tolerance study helps to provide a degree of association between a trait that characterizes salinity tolerance instead of relying only on the visual score. The traits for salinity tolerance are divided into two categories: morphological traits, and physiological traits. Both categories of traits significantly affect the productivity of cereal crops. Morphological traits are such as plant height (cm), biomass (g), panicle length (cm), and leaf area (cm$^2$) (Konaté *et al*., 2016). While physiological traits include the $K^+/Na^+$ ratio, leaf Relative Water Content (RWC), and Chlorophyll content (CHL) (Al-Ashkar *et al*., 2021). The correlation between traits can be either between morphological and physiological traits, between both morphological traits, or between physiological traits (Table 2). The knowledge of the level of correlation between traits is vital and should be prioritized in the selection process in the indirect mode (Konaté *et al*., 2016). Usually, the rice yield and grain quality decline sharply with the increase in salt concentration (Weipu, 2014; Zhang *et al*., 2022).

Zhang *et al*. (2022) found a strong ($p$ <0.05) positive correlation between potassium ion content and disintegration value in rice and protein content. But, highly ($p$<0.05) negative correlation between setback, width and length and straight chain starch content in rice flour. The salt tolerant cultivars maintain lower salt concentration in the panicle and lowest concentration in grains compared to husk and rachis. Therefore, the accumulation of mineral content under salt stress would be less and maintain the quality of rice, affecting the rice palatability.

Table 2: Summary of findings in the correlation between traits and related salinity tolerance in F3 and other populations of cereals

| Cereals | Types of Traits Components | Positive Correlation between Traits | Citations |
|---|---|---|---|
| Rice | Morphological | Dry weight of shoot & root | Amoah *et al*. (2020) |
| | Morphological | Dry weight of shoot & shoot length | |
| | Physiological | Salt injury score (SIS) & ion leakage | |
| Wheat | Physiological & morphological | $K^+/Na^+$ ratio and total chlorophyll | Dashti *et al*. (2010) |
| | Physiological & morphological | $K^+/Na^+$ ratio & number of total tillers per plant | |
| | Morphological & physiological | Height of plant & K+ content | |
| Rice | Physiological | SES score & root dry weight | Elias *et al*. (2020) |
| | Physiological | SES score & stomatal conductance | |
| | Physiological | SES score & $Na^+$ content | |

| | | | |
|---|---|---|---|
| Maize | Morphological | Plant height & yield component | Prasad *et al*. (2016) |
| | Morphological | The dry weight of the leaf & yield component | |
| | Morphological | Number of kernel rows in cob & yield component | |

*Note: Salinity Evaluation Score (SES)

### *Conventional Breeding Methods*

Plant breeders have employed the conventional breeding method to produce high-yielding and stress-tolerant crops for a long time. In terms of salinity tolerance cereal crop developments, plant breeders have succeeded in developing salt-resistant cereal crop lines or cultivars, especially for the F3 segregating line.

Several major conventional breeding methods were employed with salt tolerance traits, such as pedigree, backcrossing, and bulk breeding. Pedigree breeding involves crossing two parents, whereas commercial variety crosses with a variety with outstanding characteristics (i.e., salt tolerance). Pedigree methodology selects single plants from a cross or their offspring until they become fully homozygous (Singh *et al*., 2021). Pedigree breeding is applied in developing two segregating populations of F3 to F5 wheat (Ali, 2012) and F4 and F5 generations of wheat (Kanbar *et al*., 2011). Backcrossing methods involve crossing the variety with the donor and recurrent parent who develops backcross F1 or BC1F1 population, and the BC1F1 population is crossed to the recurrent parent for the second time (Vogel, 2009). Past studies have shown that the backcross is employed in developing oat F3 segregating generations and also F2:3 population of rice was developed from the cross Hwaseongbyeo and HG101 (Lorencetti *et al*., 2006; Yoon *et al*., 2006). According to Collard *et al*. (2017), bulk breeding comprises growing whole populations and deferring single plant selection until future generations, after which yields are evaluated. The previous studies show that bulk population was applied to F2 and F3 generation of bread wheat (El-Hendawy *et al*., 2011) and 20 segregating F3 populations of sorghums (Silva *et al*., 2018).

Despite the success of conventional plant breeding in developing the F3 and other populations of cereals with salinity tolerance attributes, there are some problems faced by the conventional breeding methods like many cereal crop species' gene pools have a small amount of genetically based diversity (Akram *et al*., 2010). Besides, Rahman *et al*. (2017) stated that conventional breeding methods are ineffective, laborious, imprecise, and time-consuming.

### *Doubled Haploid (DHs) Production*

Doubled Haploid (DHs) Production is one of the breeding methods in developing F3 cereals populations. The production of double haploids in cereal crops is from another culture. The chromosomes of the haploid were doubled using colchicine to undergo the formation of DHs. According to Singh *et al*. (2021), there are several advantages of DHs production in developing salt tolerance F3 lines, such as reducing the breeding cycle, strong selection effectiveness, homozygosity, homozygosity fixing, and also the transcription of recessive alleles appropriate for breeding. Double haploids may be an effective approach for locating QTLs that regulate quantitative characteristics. Past studies have proved the application of DHs production in the development of the F3 population of cereals with salt tolerance attributes. Lazaridou *et al*. (2013) found that the double haploid lines for barley were produced from the superior F3 plants selected at low plant density for F2 and F3 generations. Besides, double haploids also produced the selected F3 and F4 lines of wheat mutants through hybrid and conventional breeding (Kertesz *et al*., 1991).

### *Phenotypic Screening*

One of the most important prerequisites for investigating the genetics of the salt tolerance trait in the F3 population is the identification of tolerant genotypes via phenotypic screening. Establishing an effective, repeatable, and easy mass-screening technique is critical to accomplish the breeding process efficiently. Several growth stages of crops are mainly used such as seedling, vegetative, and reproductive stages, to undergo phenotypic screening. To access crops' response to salt stress, salt stress indices like Salt Stress-Responsive index (SSR) & Salt Stress Susceptibility Index (SSI) were used in the phenotypic screening.

Phenotypic screening is generally divided into two methods which are field screening and screening in hydroponics. However, from these two screening methods, field screening for salinity is regarded as the most difficult method due to environmental heterogeneity and higher spatial. Therefore, screening in hydroponics for salinity tolerance is the most effective method since it is done in controlled environmental conditions. This method is conducted at the seedling stage and involves observing the salinity stress symptoms by referring to the modified standard evaluation score (Table 3) established by International Rice Research Institute (Gregorio *et al*., 1997). Mini and Asish (2019) stated that the standard evaluation score divides genotypes into five groups: Highly tolerant, tolerant, moderately tolerant, susceptible, and severely susceptible. Table 4 summarizes the salinity reaction of cereals genotypes/lines in a salinized hydroponic system based on the previous research.

Table 3: Modified Standard Evaluation Score (SES) for visual salt injury at the seedling stage
Source: Gregorio *et al*., 1997

| Score | Observation | Tolerance |
|---|---|---|
| 1 | Normal growth, no leaf symptoms | Highly tolerant |
| 3 | Nearly normal growth, but leaf tips or few leaves whitish and rolled | Tolerant |
| 5 | Growth is severely retarded; most leaves rolled; only a few are elongating | Moderately tolerant |
| 7 | Complete cessation of growth; most leaves dry; some plants dying | Susceptible |
| 9 | Almost all plants are dead or dying | Highly susceptible |

Table 4: Salinity reaction of cereals genotypes/lines in a salinized hydroponic system

| Cereals | Genotype/Lines | Salinity Score | Salinity Tolerance (phenotype) | Citations |
|---|---|---|---|---|
| Rice | FL496 | 3.0 | Tolerant | Chunthaburee *et al*. (2015) |
| | Niewdam GS.no.00621 | 3.4 | Tolerant | |
| | Niewdam GS.no.21629 | 3.4 | Tolerant | |
| Wheat | Sakha | 1 | Highly tolerant | El-Hendawy *et al*. (2011) |
| | Thassos | 2 | Tolerant | |
| | Gemeza 7 | 2 | Tolerant | |

| | | | | |
|---|---|---|---|---|
| Rice | BRRI dhan40/ IR61920-3B-22-2-1 | 5 | Moderately tolerant | Islam *et al.* (2011) |
| | BRRI dhan28/ IR50184-3B-18-2B-1 | 7 | Susceptible | |
| | Kajalshail/ IR52713-2B-8-2B-1-2 | 3 | Tolerant | |
| Rice | PBRC × BRRI dhan-29 (Line-5, Line-7, Line-9, Line-11, Line-12, Line-18, Line-19, Line-21, Line-23, Line-25) | 5 | Moderately tolerant | Mazumder *et al.* (2019) |
| Rice | CSR10 x HBC19 | 5.2 | Moderately tolerant | Kaushik *et al.* (2003) |
| Wheat | ltay2000 | 3 | Tolerant | Oyiga *et al.* (2016) |
| | 14IWWYTIR-19 | 3 | Tolerant | |
| | UZ-11CWA-8 | 3 | Tolerant | |

### *Molecular Screening via Molecular Markers*

Salt tolerance screening is mostly done solely based on phenotypic characters, which are unreliable since environmental conditions may influence them. Therefore, molecular screening techniques via molecular markers were introduced by scientists as the most effective and efficient screening methods to detect salinity tolerance genes in cereals. Molecular markers have broad applicability in genetics and agricultural development since they deliver highly precise methods for detecting QTL associated with salt tolerance in cereals (Rao *et al*., 2022).

Several molecular markers were employed in the molecular screening, such as Simple Sequence Repeats (SSR), Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), and Cleaved Amplified Polymorphic Sequence (CAPS). However, this review study will only focus on three molecular markers: SSR, RAPD, and ISSR. According to Bonilla *et al*. (2002), salinity tolerance is a polygenic trait. Therefore the molecular markers approach for screening salt tolerance in cereal crops is regarded as the most useful screening method compared to phenotypic screening as it consumes less time and low labour-intensive.

One of the important aspects of molecular screening is QTL mapping. The QTLs mapping was employed in the molecular screening for salt tolerance in cereals to identify chromosome locations that majorly impact population variance in quantitative characteristics. The QTL mapping based on salt tolerance provides information on plant breeding improvements under salinity stress conditions. Genc *et al*. (2010) stated that the studies are employed by pyramiding the traits for salinity tolerance by using QTL mapping to increase selection productivity via a trait-based approach. Several salinity tolerance traits or indices were successfully marked using the QTL mapping approach, like $Na^{+}/K^{+}$ ratio, shoot length, mean productivity, root length, and others. Table 5 summarizes the findings on the molecular screening via QTLs mapping using several molecular markers.

Table 5: Molecular screening through QTL mapping via molecular markers for salinity tolerance in cereals

| **Cereals** | **Molecular Markers** | **Primers/Locus** | **QTL/ Indices** | **Remarks** | **References** |
|---|---|---|---|---|---|
| Barley | RAPD | bPb-9624<br>bPb-3623<br>bPb-6383<br>bPb-4576 | trRL<br>trSVL<br>trSVDW<br>Rsvil | Root length, seedling vigour, Seedling vigour by dry weight seedling vigour index length | Mwando (2021); Mwando *et al*. (2021) |
| Rice | RAPD | RM8,094<br>RM1,287<br>RM3,412<br>CP6224 | Na+<br>K+<br>Na+<br>SalToL | Na+ absorption ratio, K+ absorption ratio, Na+/K+ absorption ratio, Salt tolerance | Islam *et al*. (2011) |
| Wheat | RAPD | Opz-10 | K:Na<br>NP | K+/Na+ ratio<br>Net photosynthesis | Rahman *et al*. (2004) |
| Rice | ISSR | UBC 807<br>UBC 823<br>UBC 853 | P%, PM<br>P%, PM<br>P%, PM | Percentage polymorphism, polymorphism number | Kaushik *et al*. (2003) |
| Wheat | ISSR | HB-08<br>HB-10<br>HB-12 | UB, TAB, PIC<br>UB, TAB, PIC, P%<br>UB, TAB, PIC, P% | Unique band, total amplified bands, polymorphic information content, percentage polymorphism | Gowayed and Abd El-Moneim (2021) |
| Sorghum | ISSR | UBC-823<br>UBC-855<br>UBC-857 | P%<br>P%<br>P%, UB | Percentage polymorphism, unique band | Khalil (2013) |
| Wheat | SSR | wPt-9857<br>wPt-9951<br>cfd044<br>gwm304 | Q.tn1A<br>Q.sb2A<br>Q.chl2D<br>Q.mat5A | Number of tillers, the biomass of seedlings, the content of chlorophyll, and maturity | Genc *et al*. (2010) |
| Barley | SSR | 3665819Sa<br>3265675Db<br>3398249D | QSlsd. YG.1H<br>QSlsw. YG.2H<br>QnaKsd.1H | Salinity drain tolerance, salinity waterlogged tolerance, leaf Na/K ratio | Ma *et al*. (2015) |
| Rice | SSR | OPZ-5<br>OPZ-6<br>OPZ-11 | PH, NT, PL, GY | Plant height, number of tillers, panicle length, grain yield | Rajani and Elanchezian (2012) |

*Note: Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR), Simple Sequence Repeat (SSR), UBC (University of British Columbia (UBC)

### *Genome-Wide Association Study (GWAS) for Salt Tolerance in Cereals*

GWAS is an abbreviation for Genome-Wide Association Study. GWAS is a useful method for identifying genotype–phenotype correlations and is mostly centred on linkage disequilibrium and recombination. GWAS was applied as one of the potential screening methods because its properties are hypothesis-free and can uncover more trait-related SNPs (Yasir *et al*., 2019). In GWAS, single nucleotide polymorphisms (SNPs) are utilized as genetic markers due to the highest resolution mapping that they can achieve (Huang & Han, 2014; Burghard *et al*., 2017). Several traits were used in the GWAS for salinity tolerance, such as the number of tillers, grain length, grain yield, number of grains, $Na^{+/}K^{+}$ ratio, and others. In a study on *Oryza sativa* (rice) by Liu *et al*. (2019), GWAS analysis founds 19 different QTLs or agronomic traits after screening for salinity tolerance using 3,455,952 SNP markers. The QTLs are salt seedling survival day, seedling number, grain yield per plot, and heading date. The GWAS study conducted by Oyiga *et al.* (2018) for salinity tolerance in wheat using 90K SNP markers found two traits: salt tolerance index and the ratio of $Na^{+/}Cl^{-}$ in the leaf. GWAS analysis on barley using 3,968 SNP markers results in two traits which are $Na^{+}$ and $K^{+/}Na^{+}$ ratio (Hazzouri *et al*., 2018). A GWAS study by Xie *et al*. (2019) on maize found a result of two traits which are plant height and fresh weight, using 34,972 SNP markers for the screening process. 7 SNP loci and 8 candidate genes related to salt tolerance in maize were identified. This past study proves that GWAS are a high-potential method for screening salt tolerance cereals.

## Conclusion

This review mainly highlights the screening process for salinity tolerance in the F3 segregating population via two methods which are phenotypic screening using salinity evaluation score and molecular screenings via QTL mappings using molecular markers. Both screening methods are important in identifying germplasm with salinity tolerance attributes. This review also revealed that the selection of yield and yield components and correlation between traits associated with salinity tolerance helps develop cereal crops that not only have salinity tolerance traits characteristics but also can produce a high yield even under salinity stress conditions. However, a continuous understanding of the screening and selection process is crucial to improve and fasten the breeding process. It can be done by exploring other potential methods for screening and breeding, such as Genome-Wide Association Study (GWAS) and Double Haploid Productions.

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