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Tissue Culture-Based Strategies for Wheat (*Triticum aestivum* L.) Improvement: A Comprehensive Review

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Abstract—Wheat (*Triticum aestivum* L.) is a major staple crop essential for global food security, yet its productivity is increasingly challenged by biotic and abiotic stresses, climate change, and limitations of conventional breeding. Tissue culture-based approaches have emerged as effective tools to accelerate wheat improvement and expand genetic variability. This review highlights the role of key tissue culture techniques, including callus culture, plant regeneration, somatic embryogenesis, *in vitro* selection for stress tolerance, and genetic transformation in wheat crop improvement. Callus culture and efficient regeneration systems underpin most biotechnological applications, enabling somaclonal variation, stress screening, and genetic manipulation. *In vitro* selection has been successfully used to develop wheat lines tolerant to salinity, drought, heat, and heavy metal stress under controlled conditions. In addition, advances in genetic transformation and genome editing technologies have facilitated precise improvement of traits related to stress tolerance, disease resistance, yield stability and grain quality. Despite existing challenges, the integration of tissue culture with modern biotechnological tools offers promising prospects for developing climate-resilient and high-yielding wheat cultivars.

***Index Terms*—Tissue culture, Callus culture, Somatic embryogenesis, In vitro selection, Stress tolerance, Genetic transformation.**

1. Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops globally and plays a pivotal role in ensuring food and nutritional security. It occupies a central position in human diets by providing approximately 20 % of the total calories and protein consumed worldwide. Wheat cultivation spans diverse agro-climatic regions, ranging from temperate to subtropical environments, reflecting its broad adaptability (Shewry, 2009; FAO, 2021). However, the continuous rise in global population, shrinking arable land, climate change, and the increasing incidence of biotic and abiotic stresses pose serious challenges to sustaining wheat productivity.

Conventional wheat breeding has contributed significantly to yield enhancement during the Green Revolution; however, the rate of genetic gain has slowed in recent decades. This stagnation is attributed to the narrow genetic base of modern wheat cultivars, long breeding cycles, and the complex hexaploid genome of wheat, which complicates trait introgression and selection (Reynolds et al., 2012). Moreover, traditional breeding approaches are often inadequate for addressing complex traits such as drought tolerance, heat stress resilience, and disease resistance, which are governed by multiple genes and are highly influenced by environmental factors.

In this context, plant tissue culture techniques have emerged as indispensable tools for wheat crop improvement. Plant tissue culture is based on the principle of cellular totipotency which states that every living plant cell has the inherent capacity to regenerate into a complete plant under suitable *in vitro* conditions (Murashige & Skoog, 1962). Tissue culture enables the manipulation of plant cells, tissues, and organs under controlled environmental conditions, thereby facilitating rapid regeneration, selection, and genetic modification independent of seasonal constraints (Larkin & Scowcroft, 1981).

Among various tissue culture methodologies, callus culture and somatic embryogenesis form the foundation of *in vitro* wheat improvement. Immature embryos are the most widely used as explants due to their high embryogenic potential and responsiveness to plant growth regulators such as 2,4-dichlorophenoxyacetic acid (2,4-D) (Redway et al., 1990). These regeneration systems provide a reliable platform for inducing genetic variability, *in vitro* stress screening, and genetic transformation (Cheng et al., 1997). Successful regeneration remains a critical determinant of the efficiency of downstream biotechnological applications.

Tissue culture also facilitates the generation of somaclonal variation, which refers to heritable genetic and epigenetic changes arising during *in vitro* culture. Although somaclonal variation can sometimes lead to undesirable traits, it has been successfully exploited to develop wheat lines with improved yield attributes altered maturity duration, and enhanced resistance to diseases such as leaf rust and spot blotch (Larkin & Scowcroft, 1981; Jain, 2001). This source of novel variation is particularly valuable for crops like wheat, where conventional hybridization with wild relatives may be limited.

Another significant contribution of tissue culture to wheat

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improvement is the production of haploid and doubled haploid plants through anther or microspore culture.

Doubled haploids enable the rapid development of completely homozygous lines within a single generation, drastically reducing the time required for cultivar development and genetic analysis (Touraev et al., 2009). This approach has been widely adopted in modern wheat breeding programs to accelerate selection efficiency and variety release.

Furthermore, tissue culture is an essential prerequisite for genetic transformation and genome editing technologies**.** *Agrobacterium*-mediated transformation and biolistic methods rely on efficient regeneration systems to produce transgenic plants expressing genes for resistance to biotic stresses, tolerance to abiotic stresses, and improved grain quality (Jones, 2005). Recent advances in genome editing tools such as CRISPR/Cas systems further underscore the importance of robust tissue culture protocols for precise and targeted wheat improvement.

In summary, tissue culture approaches have become integral to modern wheat improvement strategies by enabling rapid regeneration, creation of genetic variability, and precise genetic manipulation. When integrated with conventional breeding and molecular tools, tissue culture offers sustainable solutions to overcome current productivity constraints and meet future global food demands.

A. Tissue Culture Approaches in Wheat Improvement

Plant tissue culture techniques provide a versatile platform for the genetic improvement of wheat by enabling *in vitro* regeneration, selection, and manipulation of plant material under controlled conditions. These approaches have been extensively used to overcome limitations associated with conventional breeding, such as long generation time, genotype dependency, and limited genetic variability. The major tissue culture-based approaches employed in wheat improvement are discussed below.

1) Callus Culture and Plant Regeneration

Callus culture and subsequent plant regeneration constitute the foundation of most tissue culture-based approaches used in wheat (*Triticum aestivum* L.) improvement. These processes enable the regeneration of whole plants from differentiated tissues through *in vitro* manipulation and are essential for applications such as somaclonal variation, *in vitro* selection, genetic transformation, and genome editing (Vasil & Vasil, 1980; Jain, 2001).

2) Concept and Significance of Callus Culture

Callus is an unorganized, proliferating mass of dedifferentiated cells formed when plant explants are cultured on nutrient media supplemented with appropriate plant growth regulators. The ability of wheat cells to dedifferentiate and subsequently regenerate whole plants is based on the principle of cellular totipotency (Murashige & Skoog, 1962).

In wheat improvement, callus culture serves as a manipulable cellular system that allows selection, induction of variation, and genetic modification under controlled conditions, which are often not feasible in whole plants (Redway et al., 1990).

3) Explant Sources for Callus Induction

Several explants have been used for callus induction in wheat like immature embryos, mature embryos, shoot apices, leaf bases, and inflorescence tissues. Among these immature embryos are the most widely used and highly responsive explants due to their high metabolic activity and meristematic nature (Vasil & Vasil, 1980; Redway et al., 1990).

Mature embryos are easier to obtain and store but generally show lower callus induction and regeneration frequencies compared to immature embryos (Mahmood et al., 2012). The physiological age of the explant significantly influences callus quality and embryogenic potential.

4) Media Composition and Growth Regulators

The success of callus induction and regeneration in wheat largely depends on the composition of the culture medium. Murashige and Skoog (MS) medium is the most commonly used basal medium for wheat tissue culture (Murashige & Skoog, 1962).

Auxins particularly 2,4-dichlorophenoxyacetic acid (2,4-D) are essential for callus induction. Concentrations ranging from 1–4 mg/l are commonly used to promote cell dedifferentiation (Redway et al., 1990). Cytokinins such as Benzylaminopurine (BAP) and Kinetin are required during the regeneration phase to stimulate shoot formation.

Reduction or removal of auxins and adjustment of auxin-to-cytokinin ratios trigger differentiation and regeneration (Mahmood et al., 2012). Carbon sources (sucrose or maltose), vitamins, and gelling agents also influence callus growth and regeneration efficiency.

5) Types of Callus in Wheat

Callus induced in wheat can be broadly classified into:

- *Embryogenic callus*: Compact, nodular, and cream-colored; capable of regenerating whole plants through somatic embryogenesis or organogenesis.
- *Non-embryogenic callus*: Soft, watery, and translucent; generally lacks regeneration potential (Redway et al., 1990).

The identification and maintenance of embryogenic callus are critical for successful plant regeneration and downstream applications.

6) Factors Affecting Callus Induction and Regeneration

Several factors influence the efficiency of callus culture and plant regeneration in wheat:

- *Genotype dependency*: Different wheat cultivars show wide variation in callus induction and regeneration potential (Jain, 2001).
- *Explant age and type*: Immature embryos outperform mature tissues (Redway et al., 1990).
- *Plant growth regulators*: Type, concentration, and ratio significantly affect outcomes (Mahmood et al., 2012).
- *Culture conditions*: Temperature (24-26 °C), photoperiod, and duration of culture influence morphogenesis and regeneration.

2. Plant Regeneration Pathways

Plant regeneration from callus in wheat occurs mainly through two pathways:

A. Organogenesis

Organogenesis involves the formation of shoots and roots from callus tissue in response to a specific balance of auxins and cytokinins. Shoots are induced first, followed by root formation on auxin-supplemented or hormone-free media (Mahmood et al., 2012).

B. Somatic Embryogenesis

In this pathway, somatic cells within the callus develop into bipolar embryos resembling zygotic embryos. These embryos germinate into complete plants with both shoot and root meristems (Vasil & Vasil, 1980). Somatic embryogenesis is considered more efficient and genetically uniform than organogenesis.

Continuous optimization of culture conditions and the use of molecular markers help mitigate these limitations (Jain & Gupta, 2005).

Somatic embryogenesis is a specialized *in vitro* developmental pathway in which somatic (non-reproductive) plant cells undergo dedifferentiation and redifferentiation to form bipolar embryos capable of developing into complete plants without fertilization. In wheat (*Triticum aestivum* L.), somatic embryogenesis represents a highly efficient regeneration system and serves as a cornerstone for advanced biotechnological applications such as genetic transformation, doubled haploid production, and genome editing (Vasil & Vasil, 1980; Jain et al., 2005).

C. Induction of Somatic Embryogenesis

The induction phase involves the reprogramming of somatic cells to acquire embryogenic competence. In wheat, immature embryos are the most responsive explants due to their actively dividing cells and high endogenous hormone levels (Redway et al., 1990). Mature embryos, leaf bases, and inflorescence tissues have also been used, though with comparatively lower efficiency (Mahmood et al., 2012).

Auxins, particularly 2,4-dichlorophenoxyacetic acid (2,4-D), are critical for embryogenic callus induction. High auxin concentrations promote cellular dedifferentiation and stimulate the formation of embryogenic callus. The use of Murashige and Skoog (MS) basal medium supplemented with 2,4-D (1–4 mg L^{-1}) is widely reported for wheat somatic embryogenesis (Murashige & Skoog, 1962; Vasil & Vasil, 1980). Genotype dependency is a major factor influencing embryogenic response, with certain cultivars exhibiting superior embryogenic potential (Jain, 2001).

D. Developmental Stages of Somatic Embryos

Somatic embryos in wheat follow developmental stages analogous to zygotic embryogenesis, including globular, scutellar, coleoptilar, and mature embryo stages. These embryos possess distinct shoot and root meristems, ensuring the regeneration of complete and genetically uniform plants (Jain et al., 2005).

Histological studies have confirmed the bipolar nature of wheat somatic embryos, distinguishing them from organogenic structures that lack organized meristematic regions (Vasil & Vasil, 1980). The synchronized development of somatic embryos enables large-scale propagation and uniform plant production.

E. Maturation and Germination of Somatic Embryos

The maturation phase is crucial for the successful conversion of somatic embryos into viable plantlets. Reduction or removals of auxins and supplementation with abscisic acid (ABA) have been shown to enhance embryo maturation, desiccation tolerance, and normal development (Jain & Gupta, 2005).

Partial desiccation treatments and osmotic agents such as polyethylene glycol (PEG) are often employed to improve embryo quality and germination rates. Mature somatic embryos are subsequently transferred to hormone-free or cytokinin-supplemented media for germination and shoot elongation (Mahmood et al., 2012).

F. Factors Affecting Somatic Embryogenesis in Wheat

Several factors influence the efficiency of somatic embryogenesis in wheat:

- *Explants source and physiological age*: Immature embryos show higher responsiveness than mature tissues (Redway et al., 1990).
- *Genotype*: Embryogenic competence varies widely among wheat cultivars (Jain, 2001).
- *Plant growth regulators*: Type and concentration of auxins and cytokinins significantly affect induction and regeneration (Vasil & Vasil, 1980).
- *Culture conditions*: Light, temperature, and duration of culture play crucial roles in embryo development and maturation (Jain et al., 2005).

G. Applications of Somatic Embryogenesis in Wheat Improvement

Somatic embryogenesis has numerous applications in wheat biotechnology:

- *Mass clonal propagation*: Enables rapid multiplication of elite genotypes under controlled conditions.
- *Genetic transformation*: Serves as an efficient regeneration system for transgenic wheat development (Cheng et al., 1997).
- *Somaclonal variation*: Generates novel genetic variability that can be exploited for crop improvement (Larkin & Scowcroft, 1981).
- *Germplasm conservation*: Facilitates long-term preservation through cryopreservation of embryogenic cultures (Jain et al., 2005).
- Genome editing: Essential for regenerating edited cells into whole plants using CRISPR/Cas technologies (Bhowmik et al., 2021).

3. Somaclonal Variation

Somaclonal variation refers to genetic and epigenetic

changes that arise during *in vitro* culture. Although initially considered an undesirable effect, somaclonal variation has been recognized as a valuable source of novel genetic diversity for crop improvement (Larkin & Scowcroft, 1981).

In wheat, somaclonal variants have been reported to exhibit improved agronomic traits such as early maturity, enhanced grain yield, altered plant architecture, and resistance to diseases like leaf rust and spot blotch (Jain, 2001). These variations can be stabilized through successive generations and incorporated into breeding programs.

4. *In Vitro* Selection for Stress Tolerance

In vitro selection is a tissue culture–based approach in which plant cells, calli, or regenerants are exposed to specific stress agents under controlled laboratory conditions to isolate tolerant cell lines and plants. This strategy exploits cellular totipotency and somaclonal variation**,** enabling the development of stress-tolerant wheat genotypes without the need for extensive field screening in early generations (Larkin & Scowcroft, 1981; Jain, 2001).

In wheat (*Triticum aestivum* L.), *in vitro* selection has been widely explored to improve tolerance to abiotic stresses such as salinity, drought, heat, and heavy metal toxicity, which significantly limit productivity under changing climatic conditions (Ashraf & Foolad, 2007).

A. Methodology of In Vitro Selection

The *in vitro* selection process generally involves the following steps:

1. Induction of embryogenic callus from responsive explants, typically immature embryos.
2. Exposure of callus or cell cultures to selective agents (stress factors) incorporated into the culture medium.
3. Selection of surviving or proliferating cell lines under stress conditions.
4. Regeneration of whole plants from selected calli.
5. Evaluation of regenerated plants for stress tolerance under greenhouse and field conditions (Jain, 2001; Rai et al., 2011).

This stepwise approach allows early and efficient elimination of stress-susceptible genotypes.

B. In Vitro Selection for Salinity Tolerance

Salinity stress is one of the most extensively studied abiotic stresses using *in vitro* selection in wheat. Sodium chloride (NaCl) is commonly added to the culture medium to impose salt stress. Studies have demonstrated that wheat calli selected on NaCl-supplemented media exhibit enhanced regeneration capacity and improved physiological traits such as higher proline accumulation, better ion homeostasis (Na^+/K^+ ratio), and increased antioxidant enzyme activity (Zair et al., 2003; Rai et al., 2011).

Salt-tolerant somaclones regenerated through *in vitro* selection have shown stable tolerance across generations, indicating the heritable nature of selected traits (Ashraf & Foolad, 2007).

C. In Vitro Selection for Drought Tolerance

Drought stress is simulated *in vitro* using osmotic agents such as polyethylene glycol (PEG), mannitol, or sorbitol, which reduce water availability without causing ionic toxicity. PEG-induced osmotic stress has been widely used to select drought-tolerant wheat calli. Selected lines often show improved water-use efficiency, higher relative water content, and enhanced accumulation of osmoprotectants like proline and soluble sugars (Bohnert et al., 1995; Rai et al., 2011). Such *in vitro*–selected lines provide valuable genetic material for breeding programs aimed at improving drought resilience.

D. In Vitro Selection for Heat and Temperature Stress

Heat stress tolerance in wheat is critical due to rising global temperatures. *In vitro* selection under elevated temperatures (35–40 °C) has been used to identify thermotolerant cell lines. Heat-tolerant calli often exhibit increased expression of heat shock proteins (HSPs), membrane stability, and enhanced antioxidant defense mechanisms (Wahid et al., 2007). Regenerated plants from these calli have shown improved survival and grain filling under heat stress conditions.

E. In Vitro Selection for Heavy Metal and Toxicity Tolerance

Wheat plants grown in contaminated soils face heavy metal stress from elements such as cadmium (Cd), aluminum (Al), and nickel (Ni). *In vitro* selection using toxic concentrations of these metals has enabled the isolation of tolerant cell lines. Selected calli demonstrate reduced metal uptake, enhanced sequestration, and increased activity of detoxification enzymes (Rai et al., 2011). Such approaches are useful not only for crop improvement but also for phytoremediation research.

F. Advantages of In Vitro Selection

- Rapid screening of large populations under controlled conditions
- Reduced environmental variability compared to field screening
- Early elimination of stress-susceptible genotypes
- Complementary to conventional breeding and molecular approaches

G. Limitations and Challenges

Despite its advantages, *in vitro* selection has certain limitations:

- Strong genotype dependency.
- Somaclonal variation may cause undesirable traits.
- Stress tolerance at the cellular level may not always correlate with whole-plant performance.
- Need for extensive validation under field conditions (Jain, 2001; Ashraf & Foolad, 2007).

5. Haploid and Doubled Haploid Production

The production of haploid and doubled haploid (DH) plants through another culture or isolated microspore culture represents a major breakthrough in wheat breeding. Haploid plants contain a single set of chromosomes, which can be doubled using colchicine to obtain completely homozygous

lines (Touraev et al., 2009).

Doubled haploid technology significantly shortens the breeding cycle and enhances selection efficiency. In wheat, microspore culture combined with ovary co-culture has improved embryogenesis and green plant regeneration frequencies (Zheng, 2003).

6. Genetic Transformation

Genetic transformation is a powerful biotechnological approach that enables the direct introduction of specific genes into the wheat (*Triticum aestivum* L.) genome, thereby overcoming the limitations of conventional breeding such as long generation cycles, linkage drag, and limited availability of useful genetic variability. In wheat improvement, genetic transformation has been extensively used to enhance resistance to biotic and abiotic stresses, improve grain quality, and increase yield stability under adverse environmental conditions (Jones, 2005; Bhalla, 2006). The development of efficient tissue culture and plant regeneration systems, particularly from immature embryos, has been instrumental in the success of wheat genetic transformation (Cheng et al., 1997).

A. Prerequisites for Wheat Genetic Transformation

Successful genetic transformation in wheat requires:

- A highly regenerable embryogenic callus system
- Efficient gene delivery methods
- Stable integration and expression of the transgene
- Reliable selection and regeneration of transformed plants

Genotype dependency remains a major constraint, as only certain wheat cultivars exhibit high transformation efficiency (Bhalla, 2006).

B. Gene Delivery Methods in Wheat

1) Particle Bombardment (Biolistics)

Particle bombardment, or biolistic transformation, was the first widely successful method for wheat transformation. In this technique, DNA-coated gold or tungsten particles are physically delivered into embryogenic callus or immature embryos using high-velocity microprojectiles (Vasil et al., 1992).

Advantages:

- Applicable to a wide range of genotypes
- No requirement for *Agrobacterium* host compatibility

Limitations:

- Multiple or rearranged transgene insertions
- Possible transgene silencing

Despite these limitations, biolistics remains a commonly used method for wheat genetic engineering (Altpeter et al., 2005).

2) Agrobacterium-Mediated Transformation

Agrobacterium tumefaciens–mediated transformation has become increasingly popular due to its tendency to produce fewer transgene copies and more stable gene expression. Cheng et al. (1997) reported the first successful *Agrobacterium*-mediated transformation of wheat using immature embryos. Subsequent optimization of infection conditions, co-cultivation duration, and acetosyringone supplementation significantly improved transformation efficiency (Jones, 2005).

Advantages:

- Low-copy, stable transgene integration
- Reduced gene rearrangements

Challenges:

- Strong genotype dependency
- Sensitivity of wheat tissues to bacterial infection

7. Selection and Marker Systems

Selection of transformed cells is achieved using selectable marker genes, which confer resistance to antibiotics or herbicides.

Commonly used marker systems include:

- bar gene (phosphinothricin resistance)
- nptII gene (kanamycin resistance)
- hpt gene (hygromycin resistance)

Reporter genes such as GUS, GFP, and LUC are employed to confirm gene expression and transformation efficiency (Altpeter et al., 2005).

A. Regeneration and Molecular Characterization

Following selection, transformed calli are regenerated into whole plants through somatic embryogenesis or organogenesis. Transgenic plants are subjected to molecular analyses including:

- PCR for transgene detection
- Southern blotting for copy number determination
- RT-PCR and Western blotting for expression analysis

Stable inheritance of transgenes has been demonstrated over successive generations in wheat (Jones, 2005).

B. Traits Improved through Genetic Transformation

Genetic transformation has been used to introduce a wide range of beneficial traits into wheat:

1) Abiotic Stress Tolerance

Genes related to drought, salinity, and heat tolerance, such as DREB, HVA1, and NHX, have been successfully expressed in wheat to improve stress resilience (Bhalla, 2006).

2) Biotic Stress Resistance

Transgenic wheat lines expressing Bt toxins, chitinases, and glucanases have shown enhanced resistance to insect pests and fungal diseases (Altpeter et al., 2005).

3) Quality and Nutritional Improvement

Genes influencing gluten strength, starch composition, and micronutrient content have been introduced to improve end-use quality and nutritional value of wheat grain (Shewry & Jones, 2005).

8. Conclusion

Wheat (*Triticum aestivum* L.) continues to be a cornerstone of global food security; however, its sustainable production is increasingly challenged by climate change, biotic and abiotic stresses, shrinking arable land, and the inherent limitations of conventional breeding methods (Shewry, 2009; FAO, 2021). In this scenario, tissue culture-based approaches have emerged as

indispensable components of modern wheat improvement strategies, enabling rapid, precise, and controlled manipulation of plant genetic resources.

This review comprehensively highlights the significance of tissue culture approaches, including callus culture, plant regeneration, somatic embryogenesis, *in vitro* selection, and genetic transformation, in enhancing wheat productivity and resilience. Callus culture and efficient regeneration systems form the foundation of wheat biotechnology, facilitating downstream applications such as somaclonal variation, stress screening, and genetic modification (Vasil & Vasil, 1980; Jain, 2001). Among regeneration pathways, somatic embryogenesis has proven particularly effective due to its high regeneration frequency, genetic uniformity, and suitability for large-scale propagation and transformation studies (Redway et al., 1990).

The application of *in vitro* selection has significantly contributed to the development of wheat lines tolerant to salinity, drought, heat, and heavy metal stress by enabling early-stage screening at the cellular level (Larkin & Scowcroft, 1981; Rai et al., 2011). This approach reduces dependence on extensive field trials and allows precise selection under controlled conditions, thereby accelerating the breeding process. When combined with physiological and molecular analyses, *in vitro* selection offers a robust strategy for identifying heritable stress-tolerant traits (Ashraf & Foolad, 2007).

Furthermore, genetic transformation has revolutionized wheat improvement by allowing the direct introduction of specific genes related to stress tolerance, disease resistance, yield stability, and grain quality (Jones, 2005; Bhalla, 2006). Advances in *Agrobacterium*-mediated transformation and particle bombardment have enhanced transformation efficiency and precision, offering new possibilities for targeted trait improvement in wheat (Cheng et al., 1997; Bhowmik et al., 2021).

Despite notable progress, challenges such as genotype dependency, variable regeneration efficiency, somaclonal variation, and regulatory concerns related to transgenic crops persist (Jain, 2001; Bhalla, 2006). Addressing these constraints will require continued optimization of tissue culture protocols, development of genotype-independent systems, and greater integration with molecular breeding, genomics, and phenomics approaches.

In conclusion, tissue culture–based technologies have significantly strengthened the wheat improvement pipeline and will remain central to the development of climate-resilient, high-yielding, and nutritionally enhanced wheat cultivars. The strategic integration of tissue culture with advanced biotechnological tools holds immense promise for ensuring global food security and sustainable agriculture in the face of future environmental and population pressures.

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