



Tissue culture and genetic transformation in quinoa (*Chenopodium quinoa*)—a mini-review

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Abstract

Quinoa is considered a nutrient-rich crop with immense potential to address food security, particularly in challenging environments such as saline soils and drought. Extensive genetic resources and a reference genome exist, making it a model crop. However, breeding programs and biotechnological treatments are necessary to fully utilize quinoa as a model crop and expand quinoa agriculture, as it encounters challenges when grown outside its natural regions. To expand its use, breeding programs and biotechnological tools are needed. This review examines and summarizes the tissue culture and genetic transformation efforts for quinoa to enhance its agricultural potential. Since maintaining aseptic conditions in quinoa tissue culture is critical, most of the reviewed studies surface-sterilize using sodium hypochlorite and ethanol at concentrations and exposition times that do not affect germination. For in vitro seed germination, the studies have shown that different conditions—the strength of the growth medium, photoperiod, and temperature—result in relatively high success rates of seedling cultivation. Quinoa tissue culture methods also utilize various explants and hormones to induce specific plant responses, such as callus, shoot, or root formation. However, few studies used *Agrobacterium* for stable and transient genetic transformations, with limited success. The biggest challenge appears to be regeneration from tissue culture. Further methods, including tissue-culture-independent transformation methods, are discussed here to achieve genetic transformation in quinoa.

Key message

Standardizing tissue culture data and integrating emerging biotechnologies will enhance reproducibility, enable data-driven optimization, and accelerate the genetic transformation of *Chenopodium quinoa* toward sustainable and climate-resilient crop innovation.

Keywords *Agrobacterium* · Food security · Growth regulators · Marginal soils · Micropropagation · Protoplast

Abbreviations

MASL	Meters above sea level
B5	Gamborg B5 medium
MS	Murashige & Skoog medium
LS	Linsmaier & Skoog medium
KM	Kao & Michayluk medium
KIN	kinetin

2,4-D	2,4-dichlorophenoxyacetic acid
BAP	6-benzylaminopurine
NAA	1-naphthalenacetic acid
IBA	Indole-3-butyric acid
IAA	Indole-3-acetic acid
TDZ	Thidiazuron
GA3	Gibberellic acid
2iP	6-(γ - γ -Dimethylallylamino)-Purine
AS	Acetosyringone
YEP	Yeast extract peptone medium
MES	2-morpholinoethanesulphonic acid
ZnO-NPs	Zinc oxide nanoparticles
PEG	Polyethylene glycol
OD ₆₀₀	Optical density at 600 nm
GFP	Green Fluorescent Protein

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GUS	β -Glucuronidase
VIGS	Virus-induced gene silencing
a.i	Active ingredient

Introduction

Around 29% of the worldwide population suffers from food insecurity, with rural areas being more affected than peri- and urban areas. To contrast these values, food insecurity in Africa is 68%, while in Europe it is 8.7% (FAO et al. 2024). Related to this, the number of undernourished people globally reached 9.1% in 2023 (FAO et al. 2024), a problem expected to worsen with population growth (Hall et al. 2017). Since most food calories—directly or indirectly—come from agricultural lands (Cassidy et al. 2013), agricultural areas are well known to support food security (Avtar et al. 2020). To continue doing so, the current yield growth rate has to increase by 37% to cope with the food demand of 2050 (Tester and Langridge 2010). A strategy to increase yield could be to utilize marginal environments, such as underutilized saline soils, with suitable crops like quinoa (*Chenopodium quinoa*, Willd.) (Schmöckel et al. 2017).

From the Amaranthaceae family, quinoa is an ancient crop from the Andean region of Peru and Bolivia (Vega-Gálvez et al. 2010); adapted to high altitudes (>3500 MASL), and tolerant to many abiotic stresses (Adolf et al. 2012). Quinoa is a highly nutritional seed crop (Repo-Carrasco et al. 2003), gluten-free by nature, with high protein content, all essential amino acids, minerals, vitamins, dietary fiber, unsaturated fatty acids, and other beneficial compounds (Vega-Gálvez et al. 2010; Tang et al. 2015). Due to its adaptability and nutritional value, quinoa's potential has caught the attention of the United Nations, which declared 2013 as the International Year of Quinoa (United Nations 2012).

Despite its vast agronomical potential, quinoa is not well adapted to regions outside its origin—including tropical lowlands and subtropical environments. In addition, it presents seed shattering, pre-harvest sprouting, lodging, and susceptibility to novel diseases, all of which are undesirable traits for modern agricultural systems (Murphy et al. 2019). Therefore, breeding efforts are necessary to improve important agronomic traits to boost quinoa production on a global scale (Jarvis et al. 2017). Those breeding programs could profit not only from the available reference genome (Jarvis et al. 2017; Rey et al. 2023), but also from biotechnological tools such as tissue culture, genetic transformation, and genome editing, to first understand specific mechanisms (e.g., tolerance to abiotic stress) and ultimately to achieve breeding objectives (Regalado et al. 2020).

Based on tissue culture, plant biotechnology enables the manipulation of plant development and growth in

laboratory-controlled conditions. This technique is essential to develop enhanced varieties, preserve species, and produce plant compounds (Baria et al. 2024). Besides, obtaining homozygous plants is faster compared to conventional breeding methods (Hussain et al. 2012), and the multiplication rate from a single explant can yield up to millions of plants in one year. This massive production of plants, known as micropropagation, is promising for conservation, research, and commercial use (Baria et al. 2024). Given its importance, it is imperative to establish a micropropagation protocol for quinoa (Regalado et al. 2020).

Due to its short life cycle and salt tolerance, quinoa is a suitable model crop for studying salt tolerance, a complex trait that is challenging to investigate in other model plants (Jacobsen et al. 2015; Wang et al. 2023). Developing genetic manipulation methods to study gene function is crucial for understanding the unique pathways of quinoa; however, only two stable transformation protocols have been reported for quinoa (Sidorov et al. 2024; Bu et al. 2025). Therefore, this document aims to gather, explore, and summarize tissue culture efforts on quinoa, including seed sterilization, in vitro germination, morphogenesis, callus formation, somatic embryogenesis, and genetic transformation protocols published to date, and discuss these in light of other species transformations and future opportunities.

Materials and methods

Papers to be reviewed were retrieved from Google Scholar using the following search key: allintitle:"quinoa" (micropropagation OR regeneration OR propagation OR organogenesis OR callogenesis OR "in vitro culture" OR "somatic embryogenesis" OR transformation OR "plant transformation" OR "callus" OR "calli" OR "gene editing" OR "genome editing" OR "genome engineering" OR "CRISPR" OR "Cas9") -intitle:"review". The search was done at "Any moment" (until September 12, 2025), and the 26 results (excluding citations and patents) were exported from Google Scholar. Peer-reviewed papers and chapters in English with available PDFs were reviewed, while unrelated documents, theses, repeated papers, and preprints were excluded from the review, resulting in 17 papers.

With the dataset compiled from the in vitro culture studies reviewed in this work, a network diagram was constructed in RStudio using the *ggplot2*, *tidyverse*, and *GGally* packages. The network includes the reported combinations of regeneration pathways, growth regulators, and explant types used in in vitro quinoa callogenesis and organogenesis experiments. Nodes represent these categories, with size scaled according to the number of reports (for pathways) or the mean concentration (for regulators). Edge color

indicates the regeneration step (purple for Organ induction and orange for Callus induction).

Surface sterilization protocols in quinoa

When working with plant tissue culture, maintaining sterility and an aseptic environment are essential for conducting experiments (Varma and Jain 2021). Most initial tissues in quinoa tissue culture are excised from sterile seedlings germinated *in vitro*; therefore, most studies report successful seed surface sterilization (Fig. 1). The most common sterilization agent reported for quinoa is sodium hypochlorite (NaOCl) after treatment with ethanol. These protocols require the lowest concentration of the sterilization agent for the shortest possible time, followed by rinsing the seeds several times with sterile water to remove the residual sterilization agent (Varma and Jain 2021).

While sterilization protocol methods vary significantly in terms of treatment concentration and exposure time, they were the only or most effective method reported by the authors, resulting in higher germination rates and lower contamination (Table 1). Some protocols include a long soak in water (several hours), presumably to reduce the amount of saponins on the seed and make the seed amenable to sterilization agents.

One of the first methods published was the surface sterilization of seeds from varieties Blanca de Junin and Real de Puno with 10% calcium hypochlorite ($\text{Ca}(\text{OCl})_2$) for 20 min, eliminating the excess of $\text{Ca}(\text{OCl})_2$ with two rinses

of sterile water (Burnouf-Radosevich and Paupardin 1985). Wang et al. (2023) sterilized seeds from cultivars MS509/N, QN108B, 141R, and 20ALC by exposure to chlorine gas (Cl_2 , produced by mixing HCl and NaOCl) inside a desiccator for 4–6 h. Sidorov et al. (2024) sterilized QQ74 (PI 614886) quinoa seeds with 20% NaOCl for 20 min, followed by rinses with sterile water. Similarly, Sağlam Yilmaz and Doğanci (2018) sterilized Limtar red seeds with 5% NaOCl for 5–10 min, and Shahin (2019) cleaned the seeds with 0.003% Tween-20 and rinsed them with tap water. Then, the seeds were sterilized with 20% NaOCl for 20 min, followed by three rinses of sterile water for 15 min each. Regalado et al. (2020) sterilized quinoa seeds of varieties Cahuil and Villa by immersion in NaOCl (25 g/L) for 10 min inside a vacuum chamber, finalizing with three rinses of sterile water of 5 min each.

Another study reports that quinoa seeds from different varieties—BGQ-174, BGQ-77, POQ-132, and Blanca de Junin—were first cleaned with 1% liquid detergent for 10 min, followed by 70% ethanol for 30 s. They tried different concentrations of NaOCl combined with 0.2% Tween-20 for different immersion times, with NaOCl (1.8% a.i) for 30 min giving the best sterilization result (Henarejos-Escudero et al. 2018). The remaining research reported the use of ethanol and NaOCl with different concentrations and sterilization times of seeds of unknown varieties (Eisa et al. 2005; Hesami and Daneshvar 2016; Hesami et al. 2018; Al Gethami and El Sayed 2020b), Faro (Al Gethami and El Sayed 2020a), Qingqua I (Xie et al. 2025) and stems of the Q-37 variety (Telahigue and Toumi 2017).

Fig. 1 Graphical representation of the current status of *in vitro* culture in *C. quinoa*, indicating key processes, advancements, and challenges that are further described in the corresponding tables. Figure created using biorender

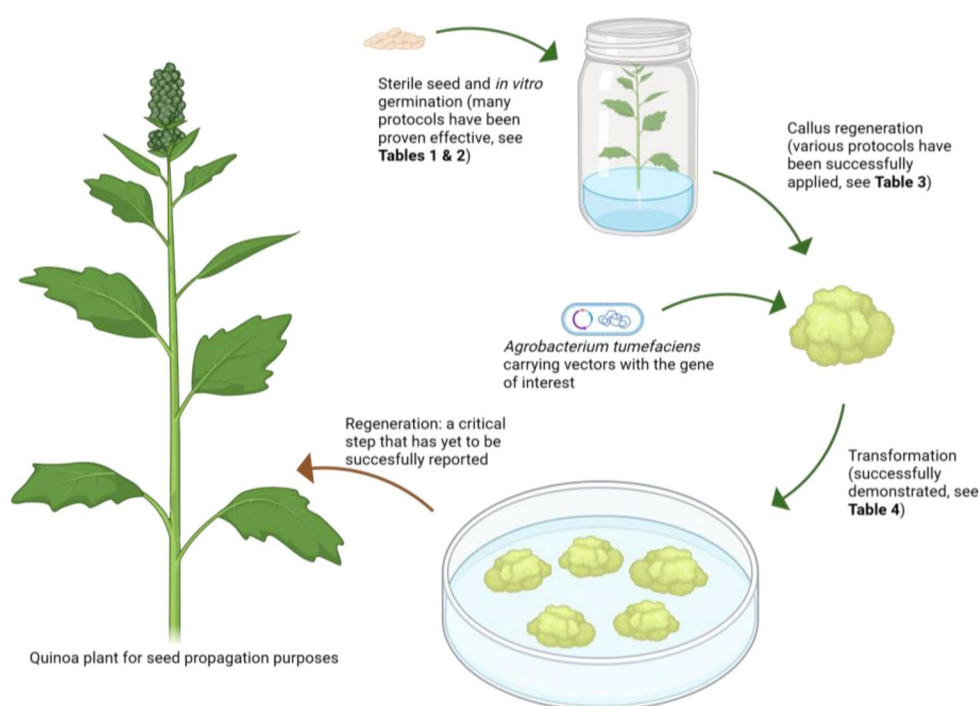


Table 1 Published protocols for in vitro establishment of quinoa: varieties, explant types, and sterilization methods

Variety	Explant	Protocol	Reference
Blanca de Junin & Real de Puno	Seeds	Surface sterilization with $\text{Ca}(\text{OCl})_2$ (10%, 20 min)	Burnouf-Radosevich and Paupardin (1985)
Not indicated	Seeds	Surface sterilization with ethanol (70%, 5 min) and NaOCl (0.6% a.i., 30 min)	Eisa et al. (2005)
Not indicated	Seeds	Surface sterilization with ethanol (70%, 15 s) and NaOCl (0.525% a.i., 10 min)	Hesami and Daneshvar (2016)
Q-37	Stems (5 cm)	Surface sterilization with NaOCl (50%, 30 min) and ethanol (70%, 5 min)	Telahigue and Toumi (2017)
Not indicated	Seeds	Surface sterilization with ethanol (70%, 15 s) and NaOCl (20%, 5 min)	Hesami et al. (2018)
BGQ-174, BGQ-77, POQ-132, Blanca de Junin	Seeds	Surface sterilization with commercial liquid detergent (1%, 10 min), ethanol (70%, 30 s), and NaOCl (1.8% a.i.) + Tween 20 (0.2%) for 30 min	Henarejos-Escudero et al. (2018)
Limtar red	Seeds	Surface sterilization with NaOCl (5%, 5–10 min)	Sağlam and Doğanci (2018)
Not indicated	Seeds	Surface sterilization with Tween 20 (0.003%) and NaOCl (60%, 20 min)	Shahin (2019)
Cahuil, Villa	Seeds	Surface sterilization with NaOCl (25 g/L, 10 min) in a vacuum chamber	Regalado et al. (2020)
Faro	Seeds	Surface sterilization with ethanol (70%, 15 s) and NaOCl (10%, 20 min)	Al Gethami and El Sayed (2020a)
Not indicated	Seeds	Surface sterilization with ethanol (70%, 15 s) and NaOCl (10%, 20 min)	Al Gethami and El Sayed (2020b)
MS509/N, QN108B, 141R & 20ALC	Seeds	Surface sterilization with Cl_2 in a desiccator for 4–6 h	Wang et al. (2023)
QQ74	Seeds	Surface sterilization with 20% NaOCl (20 min)	Sidorov et al. (2024)
Qingqua I	Seeds	Surface sterilization with ethanol (75%, 1 min) and NaOCl (10%, 20 min)	Xie et al. (2025)

Similarly, for other species of the Amaranthaceae family, different sterilization agents have also been used for the surface sterilization of seeds. For example, ethanol followed by HgCl_2 has been used for surface sterilization of *Amaranthus tricolor* (Tahir and Mathew 2021) and sugar beet (Zhang et al. 2023). HgCl_2 alone has been used to sterilize the axillary branches of *Chenopodium ambrosioides* (Jan et al. 2023), followed by thorough removal of the sterilization agent afterward. Another sterilization agent used is NaOCl in *C. murale* (Milivojević et al. 2005; Mitić et al. 2012), *Oxybasis rubra* (Milivojević et al. 2005), and waterhemp (Kumam et al. 2024). NaOCl has also been used in conjunction with previous sterilization methods, such as ethanol, in *A. dubius* (Areington et al. 2024) and spinach (Bao et al. 2009; Zhang and Zeevaart 1999). In this sense, several agents can be used for surface sterilization of quinoa seeds with acceptable efficiency. In our experience, surface sterilization with 1% (a.i) NaOCl (5–20 min), a washing step of 5 min with sterile water, followed by 70% ethanol (5 min), and drying in a laminar flow hood is sufficient to obtain sterile seeds suitable for in vitro germination.

In vitro germination protocols in quinoa

In vitro seed germination is a complex process (Genze et al. 2020) influenced by both biological and physical factors (e.g., pH, temperature, and light), as well as the sterilization protocol and composition of the medium (Hesami et

al. 2021). In this sense, the medium used for germination, incubation conditions, and sterilization protocols could affect the in vitro germination of quinoa seeds (Table 2). Quinoa seed germination appears highly efficient under various conditions (Fig. 1), whether on MS medium or moist paper, regardless of whether it is under dark or light conditions. Based on our experience, germination rates decrease after long-term seed storage (lasting more than two to three years).

For the first germination protocol described here, germination was done on sterile plates under a 12-hour photoperiod at 22 °C (it remains unclear which salts were used and which germination efficiency was obtained) (Burnouf-Radosevich and Paupardin 1985). Eisa et al. (2005) did not report the germination efficiency of their germination performed on MS medium (Murashige and Skoog 1962) under a 16-hour photoperiod at 25 °C.

However, a germination efficiency of 100% can be achieved on MS salts at 26 °C in the dark (Hesami and Daneshvar 2016), under a 16-hour photoperiod for Limtar Red seeds (Sağlam and Doğanci 2018), or at 25 °C with a 16-hour photoperiod (Hesami et al. 2018; Shahin 2019). Germination is also successful on half-strength MS and moist paper (Henarejos-Escudero et al. 2018). Regalado et al. (2020) performed seed germination on MS for three days in the dark and then four days under a 16-hour photoperiod at 25 °C. The germination efficiency for the variety Cahuil was 95%, and for Villa was 68%. Similar to Burnouf-Radosevich and Paupardin (1985); Al Gethami and El Sayed

Table 2 Reported germination protocols for quinoa seeds: varieties, culture media, and growth conditions

Variety	Culture medium	Light (hours/day)	Temperature (°C)	Germination (%)	Reference
Blanca de Junin, Real de Puno	Not indicated	13.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ * (12)	22	Not indicated	Burnouf-Radosevich and Paupardin (1985)
Not indicated	MS	(16)	25	Not indicated	Eisa et al. (2005)
Not indicated	MS	Darkness	26	100	Hesami and Daneshvar (2016)
Not indicated	MS	65 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (16)	25	100	Hesami et al. (2018)
BGQ-174, BGQ-77, POQ-132, Blanca de Junin	0.5 MS Moist paper	Darkness [8 days], then photoperiod (16)	23 (day), 18 (night)	Not indicated	Henarejos-Escudero et al. (2018)
Limtar red	MS	(16)	24	100	Sağlam and Doğanci (2018)
Not indicated	MS	30 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (16)	26	100	Shahin (2019)
Cahuil	MS	Darkness [3 days], then 65 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (16) [4 days]	25	95	Regalado et al. (2020)
Villa				68	
Faro	MS+2 mg/L ZnO-NPs	13.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ * (12)	25	22	Al Gethami and El Sayed (2020a)
Not indicated	MS	13.5 $\mu\text{mol m}^{-2}\text{s}^{-1}$ * (12)	25	Not indicated	Al Gethami and El Sayed (2020b)
MS509/N, QN108B, 141R & 20ALC	0.5 MS	50 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (16)	25	Not indicated	Wang et al. (2023)
QQ74	DKW	80 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (16)	25	Not indicated	Sidorov et al. (2024)
Qingqua I	MS	27–30 $\mu\text{mol m}^{-2}\text{s}^{-1}$ * (16)	25	Not indicated	Xie et al. (2025)

*Assuming 74 lx = 1 $\mu\text{mol m}^{-2}\text{s}^{-1}$ (Islam et al. 2024)

(2020b); Al Gethami and Sayed (2020a) germinated seeds under a 12-hour photoperiod on MS alone (Al Gethami and El Sayed 2020b) or supplemented with 2 mg/L ZnO-NPs (Al Gethami and El Sayed 2020a) at 25 °C. The germination efficiency obtained in this protocol was 22%, the lowest so far (Al Gethami and El Sayed 2020a). Wang et al. (2023) used a half-strength MS medium to germinate their seeds under a low-light 16-h photoperiod at 25 °C. Sidorov et al. (2024) germinated seeds on DKW medium (Driver and Kuniyuki 1984), supplemented with 20 g/L sucrose under 16-hour photoperiod at 25 °C. Although the authors did not report germination efficiency, the plants developed inflorescence after one month, and they were used as explants to induce flowers in vitro. Lastly, Xie et al. (2025) germinated Qingqua I seeds on MS supplemented with 30 g/L sucrose under a 16-hour photoperiod 25 °C. The authors did not report germination efficiency, but hypocotyls from seedlings were used to induce callus.

The germination of different species from the Amaranthaceae family has also been studied on MS media, including *A. tricolor* (Tahir and Mathew 2021), *O. rubra*, and *C. murale* (Milivojević et al. 2005) with variations in light and temperature. Spinach seeds are germinated on MS under an 8-hour photoperiod at 23 °C (Zhang and Zeevaart 1999), while the germination of *C. ambrosioides* was performed on MS under 16-hour photoperiod at 26 °C (Jan et al. 2023). Sugar beet seeds were germinated on agar at 23 °C (Zhang et al. 2023). Meanwhile, *C. murale* seeds were germinated on moist paper, switching temperature and light regimes

for five days (Mitić et al. 2012). In quinoa, the combination of MS, 25 °C, and a 16-hour photoperiod is the most commonly used condition for in vitro seed germination, which is consistent with our experience.

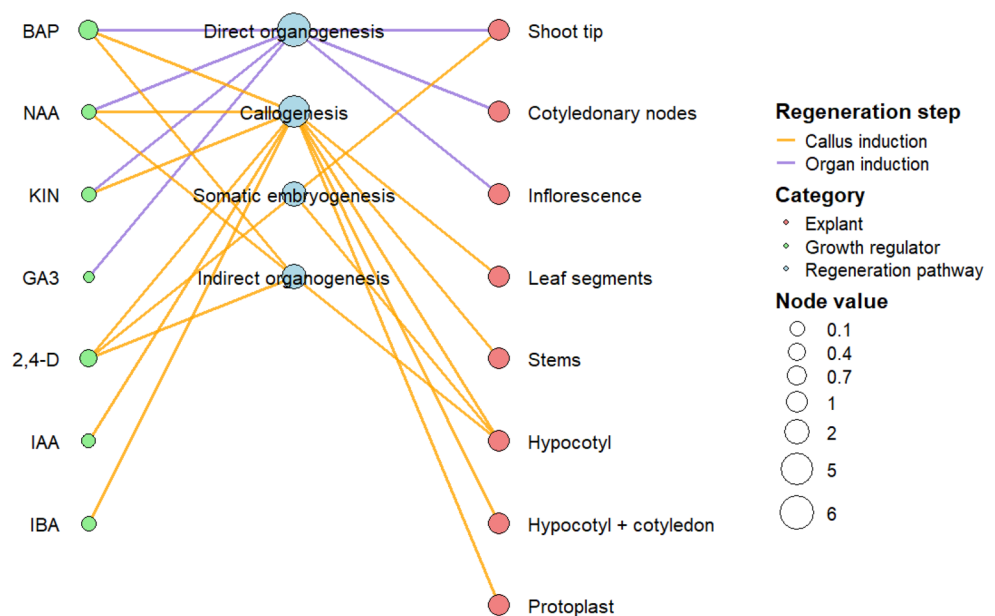
Tissue culture protocols in quinoa

Growing and maintaining plants in tissue culture requires a medium that contains macro- and micronutrients, vitamins, carbon sources, and a solidifying agent. The addition of plant hormones and their concentrations depend on the experiment's objective. Among them, auxins—2,4-dichlorophenoxyacetic acid (2,4-D), 1-naphthalenacetic acid (NAA), indole-3-butyric acid (IBA), indole-3-acetic acid (IAA)—and cytokinins—6-benzylaminopurine (BAP), kinetin (KIN), thidiazuron (TDZ)—are added individually or together to induce different responses (Varma and Jain 2021). Direct and indirect organogenesis, callogenesis, and embryogenic responses (Fig. 1) have been observed in quinoa using different types of explants (Fig. 2), as summarized in Table 3.

Callogenesis in quinoa

The callogenesis regeneration pathway in vitro typically relies on the ratio of auxins and cytokinins, and various types of calli can be obtained depending on the research purpose. The obtained calli could regenerate into organs

Fig. 2 Network diagram summarizing relationships among regeneration pathways, growth regulators, and explant types reported in in vitro quinoa regeneration studies. Node size represents the number of reports (for pathways) or mean concentration (for regulators). Edge color indicates the regeneration step (purple=Organ induction; orange=Callus induction). Figure created in RStudio using *ggplot2*, *tidyverse*, and *GGally* packages



(shoots, roots, or somatic embryos) or could not regenerate at all, being friable or compact (Ikeuchi et al. 2013). In quinoa, five papers report callogenesis in vitro (Fig. 2), either for genetic transformation (Komari 1990), secondary metabolites production (Henarejos-Escudero et al. 2018; Shahin 2019), to test the effect of water stress on callus (Telahigue and Toumi 2017) or unsuccessful regeneration from protoplast-derived callus (Sidorov et al. 2024).

Sidorov et al. (2024) isolated protoplasts from homogenized inflorescence cultures in 30 mL W5 medium (5–8 s, 20000 rpm). The homogenized material was sieved, washed with W5 medium, dried on sterile paper, and transferred to Petri dishes. The cell wall was removed with 2% Cellulase RS, 2% Cellulysin, 0.5% Driselase, and 0.5% Macerozyme R-10 for 3 h at 28 °C. After isolation, protoplasts were embedded in an alginate layer with a modified KM medium (Kao and Michayluk 1975), supplemented with 72 g/L glucose, 1 mg/L NAA, 1 mg/L BAP, and 0.25 mg/L 2,4-D (Sidorov et al. 2022). After dissolving alginate, protoplast colonies were cultured in MS medium (with B5 vitamins), supplemented with 32 g/L mannitol, 10 g/L sucrose, 3 mg/L BAP, and 0.01 mg/L IAA. One month later, colonies were transferred to a solid MS medium supplemented with 32 g/L mannitol, 10 g/L sucrose, 1 mg/L BAP, 1 mg/L KIN, and 0.2 mg/L IBA. Although the colonies grew fast on this medium, no regeneration was obtained, even after transferring the callus to media with different combinations of growth regulators. The addition of proline could help alter the callus texture to promote regeneration, as observed by Xie et al. (2025) in hypocotyl-derived callus from quinoa.

To perform genetic transformation, Komari (1990) induced callus from leaf segments on LS medium (Linsmaier and Skoog 1965) supplemented with 30 g/L sucrose,

3 mg/L IAA, 3 mg/L NAA, and 0.1 mg/L KIN. The same medium was used to maintain the callus, with subcultures every three weeks, and to prepare a suspension for the callus, with weekly subcultures. Since the main objective of the research was the transformation of the cells in suspension, the researchers did not report the environmental conditions (photoperiod, temperature) of the cultures, the variety used, the callogenesis efficiency, or regeneration from cellular suspension. The lack of this information affects the reproducibility of the protocol and limits the scope of the research.

Telahigue and Toumi (2017) induced callus from 5 cm young stems (internodes) on MS or B5 (Gamborg et al. 1968), supplemented with 0.2 mg/L BAP and 0.5 mg/L 2,4-D. The calli were induced under a 16-hour photoperiod at 25 °C. According to the researchers, 100% of explants produced friable callus. However, the largest calli with light- or dark-green color were obtained on MS medium (1.95 mm diameter). Another objective of the researchers was to test the effect of PEG 6000-induced water deficit (osmotic stress) on different callus parameters. However, to achieve this, they used MS medium supplemented with 0.2 mg/L BAP, which produced green, friable calli of 1.53 mm in diameter with 100% efficiency. Again, regeneration from the cellular suspension was out of the scope of the research.

To produce secondary metabolites, Henarejos-Escudero et al. (2018) induced callus from leaf segments (1 × 1.5 cm, 20 days old) and eight-day-old hypocotyls using 1.5 MS supplemented with 45 g/L sucrose, 1.5 mg/L BAP, and 1.5 mg/L 2,4-D. Calli were induced under a 16-hour photoperiod at 23 °C during the day and 18 °C during the night. This treatment achieved the highest cell growth, producing green, friable calli; however, efficiency was not reported.

Table 3 Regeneration pathways in quinoa tissue culture: varieties, explants, culture media, and reported outcomes

Variety	Regeneration pathway	Regeneration step	Explant	Explant age (days)	Culture medium	Growth regulator	Efficiency	Proliferation rate	Reference
Blanca de Junin	Direct organogenesis	Shoot induction	Shoot tips	6	B5	0.22 mg/L BAP+0.018 mg/L NAA	67%	3.78 shoots/explant	Burnouf-Radosevich and Paupardin (1985)
		Shoot multiplication	Induced shoots	21	B5 (modified)	0.22 mg/L BAP+0.018 mg/L NAA	Not indicated	8.46 shoots/culture	
		Rooting	Elongated shoots	730	B5 (modified)	-	75% (20 °C at day, 4 °C at night)	Not indicated	
Not indicated	Callogenesis	Callus induction	Leaf segments	Not indicated	LS	3 mg/L IAA+3 mg/L NAA+0.1 mg/L KIN	Not indicated	Not indicated	Komari (1990)
Not indicated	Indirect somatic embryogenesis	Callus induction	Hypocotyl (0.5–1 cm)	7	MS (modified)	0.1 mg/L 2,4-D	92%	Not indicated	Eisa et al. (2005)
	Indirect somatic embryogenesis	Embryo induction	Callus	30	Modified MS (liquid or solid)	-	5% [conversion rate]	Not indicated	
Not indicated	Indirect organogenesis	Callus induction	Hypocotyl (0.5–1 cm)	8–10	MS	0.5 mg/L 2,4-D+0.05 mg/L BAP	93%	1.2 g/callus	Hesami and Daneshvar (2016)
Q-37	Indirect organogenesis	Shoot induction	Callus	30	MS	1 mg/L BAP+1 mg/L KIN+0.2 mg/L IBA	86%	6.3 shoots/explant	
	Callogenesis	Callus induction	Stems (fragments of internodes, 5 cm)	Not indicated	MS or B5	0.2 mg/L BAP+0.5 mg/L 2,4-D	100% [friable]	Not indicated	Telaghue and Touni (2017)
Not indicated	Direct organogenesis	Shoot induction	Cotyledonary nodes (0.5–1 cm)	30	MS	2 mg/L BAP	93% (4.96 shoots/explant)	4.96 shoots/explant	Hesami et al. (2018)
BGQ-174, BGQ-77, POQ-132 & Blanca de Junin	Callogenesis ^a	Callus induction	Hypocotyl	8	1.5 MS	1.5 mg/L BAP+1.5 mg/L 2,4-D	Not indicated [friable callus]	Not indicated	Henarejos-Escudero et al. (2018)
BGQ-174, BGQ-77, POQ-132 & Blanca de Junin	Callogenesis ^a	Callus induction	Leaf segments	20	1.5 MS	1.5 mg/L BAP+1.5 mg/L 2,4-D	Not indicated [friable callus]	Not indicated	
Limtar red	Indirect somatic embryogenesis	Callus induction	Shoot tips	Not indicated	MS	0.5 mg/L 2,4-D	100%	Not indicated	Sağlam and Doğanç (2018)
Not indicated	Callogenesis ^a	Callus induction	Hypocotyl (0.5–1 cm)+cotyledon (0.5 cm ²)	10	MS	2 mg/L 2,4-D+0.05 mg/L KIN	95%	3.6 g/callus	Shahin (2019)

Table 3 (continued)

Variety	Regeneration pathway	Regeneration step	Explant	Explant age (days)	Culture medium	Growth regulator	Efficiency	Proliferation rate	Reference
Cahuil	Direct organogenesis	Shoot induction	Shoot tips (3 mm)	7	MS	1 mg/L KIN + 1 mg/L BAP	93%	Not indicated	Regalado et al. (2020)
		Shoot multiplication	Induced shoots	30	MS	2 mg/L BAP	Not indicated	7.96 shoots/explant	
		Rooting	Elongated shoots	30	MS	2 mg/L IBA	87%	4.12 root/shoot	
Villa	Direct organogenesis	Shoot induction	Shoot tips (3 mm)	7	MS	1 mg/L KIN + 1 mg/L BAP	79%	Not indicated	
		Shoot multiplication	Induced shoots	30	MS	2 mg/L BAP	Not indicated	4.10 shoots/explant	
		Rooting	Elongated shoots	30	MS	2 mg/L IBA	83%	4.52 roots/shoot	
Faro	Direct organogenesis	Shoot induction	Cotyledonary nodes (0.5–1 cm)	15	MS	-	93%	4 shoots/explant	Al Gethami and El Sayed (2020a)
		Rooting	Induced shoots	30	0.5 MS + 10 mg/L ZnO-NPs	-	100%	4.8 roots/shoot	
		Shoot induction	Cotyledonary nodes (0.5–1 cm)	15	MS + 10 g/L sucrose	-	93.3%	4 shoots/explant	
Not indicated	Direct organogenesis	Shoot induction	Cotyledonary nodes (0.5–1 cm)	15	MS + 10 g/L sucrose	-	60%	0.8 roots/shoot	Al Gethami and El Sayed (2020b)
QQ74	Direct organogenesis	Rooting	Induced shoots	30	0.5 MS	1 mg/L KIN + 0.5 mg/L BAP + 0.1 mg/L GA3	Not indicated	Not indicated	Sidorov et al. (2024)
		Flower formation	Inflorescence	30	DKW	3 mg/L BAP + 0.01 mg/L IAA	Not indicated	Not indicated	
		Colony induction	Protoplast	Not indicated	MS (B5 vitamins)	1 mg/L BAP + 1 mg/L KIN + 0.2 mg/L IBA	Not indicated	Not indicated	
Qingqua I	Indirect organogenesis	Callus induction	Protoplast-derived colony	30	MS (MS vitamins)	0.2 mg/L BAP + 2 mg/L NAA	97.78%	Not indicated	Xie et al. (2025)
		Callus induction	Hypocotyls (3–4 mm)	3–5	MS	1 mg/L BAP + 0.1 mg/L NAA + 0.2 g/L proline	89.63%	40.6 shoots/explant	
		Shoot induction	Callus	42	MS	0.1 mg/L BAP + 0.01 mg/L NAA	88.76%	6.85 cm/shoot	
		Shoot elongation	Induced shoots	56	3MS	-	-	-	
		Rooting	Elongated shoots	35	MS	0.6 mg/L IBA	98.52%	16.9 roots/explant	

a: Pigments/secondary metabolites production

The calli were subcultured twice at monthly intervals and then transferred to a modified medium to induce pigment accumulation. The modification included a 10-fold reduction in nitrogen and an increased BAP concentration to 2 mg/L. After 20 days, coloration was visible in the calli, except those from the Blanca de Junin variety. The calli were subcultured monthly, five times before the preparation of extracts to identify pigments, although regeneration from callus was not within the scope of the research.

To produce tocopherols, Shahin (2019) induced callus from hypocotyl (0.5–1 cm) and cotyledon (0.5 cm²) excised from 10-day-old seedlings using MS medium supplemented with 2 mg/L 2,4-D and 0.05 mg/L KIN. The cultures were maintained at 25 °C in darkness for four weeks and then transferred to light for an additional two weeks before being weighed. The callus induction efficiency obtained from both explants was 95%, with fresh calli weighing 3.6 g on average, although no regeneration from callus was performed, similar to Henarejos-Escudero et al. (2018). Finally, tocopherols were extracted from dry seeds and freeze-dried material, including calli or leaves. Similar to Komari (1990), Shahin (2019) do not report the variety used in this paper, limiting reproducibility.

The callogenesis reports in quinoa are heterogeneous in terms of the growth regulators used and the type of explant. BAP, NAA, KIN, 2,4-D, IAA, and IBA have been used to induce callus from leaf segments, stems, hypocotyl, hypocotyl+cotyledon, and protoplast (Fig. 2). Most of the methods report the use of MS to induce callus, except for Komari (1990), who used LS. In general, the explants used were relatively young, ranging from eight to 30 days old, and the induction time varied from 60 days (Telahigue and Toumi 2017) to 150 days (Henarejos-Escudero et al. 2018). Since plant regeneration is not the main objective of the researchers (Komari 1990; Telahigue and Toumi 2017; Henarejos-Escudero et al. 2018; Shahin 2019) or plant regeneration from callus is unsuccessful (Sidorov et al. 2024), no regeneration was reported. Related to this, most researchers do not report efficiency in callus induction, except for Telahigue and Toumi (2017) and Shahin (2019). At the same time, Komari (1990) and Shahin (2019) do not specify the variety used in their methods, which limits the reproducibility of the protocol.

In Amaranthaceae, the regeneration of plants from friable callus, formed from mesophyll-derived protoplasts, was possible in sugar beet using BAP or TDZ (Lenzner et al. 1995). In quinoa, even if protoplast isolation and transfection as a research tool have been broadly used (De Varennes et al. 1984; Hans et al. 1992; Jung et al. 1992; Li et al. 2022; Sun et al. 2017), this is the first time a callus has been obtained from protoplasts (Sidorov et al. 2024). Although this protoplast-derived callus from Sidorov et al. (2024)

was unable to regenerate plants, the tool can still be used to obtain transient and stable-transformed callus to test vectors of interest or try transgenes, for example, in callus studies or to produce secondary metabolites, as done by Henarejos-Escudero et al. (2018) and Shahin (2019). Related to this, in *Amaranthus* species and *Celosia argentea*, secondary metabolites were isolated from cell suspensions initiated from hypocotyl-derived callus induced on MS supplemented with BAP and 2,4-D (Xuan et al. 2023; Sang et al. 2024). In *O. rubra*, betalains were produced from cell suspensions initiated from meristem-derived callus, which was also induced on MS supplemented with 2,4-D and KIN (Berlin et al. 1986).

Using callus-derived cellular suspensions to study the effect of stressors, such as the use of PEG to induce osmotic stress (Telahigue and Toumi 2017), was tried before in Amaranthaceae. For *O. rubra*, photoautotrophic cell suspensions were induced from hypocotyl-derived callus (Hüsemann et al. 1977; Ehness et al. 1997). The cell suspension cultures in this case were exposed to different stress elicitors, including endothal and chitosan, to study stress-related signaling pathways (Ehness et al. 1997). In this regard, the use of callus or cellular suspension may be beneficial not only for biotic or abiotic stress experiments but also for cellular physiology studies.

Indirect somatic embryogenesis in quinoa

The process by which somatic cells differentiate into somatic embryos, resembling zygotic embryos, is called somatic embryogenesis. When somatic embryos are obtained from callus, the process is referred to, for convenience, as indirect somatic embryogenesis (Von Arnold et al. 2002). This embryogenic callus is an ideal target for transformation due to its high regeneration rate, stable proliferation, tolerance to *A. tumefaciens*, and sensitivity to antibiotics (Song et al. 2020). In quinoa, only two papers report somatic embryogenesis (Fig. 2) (Eisa et al. 2005; Sağlam and Doğancı 2018).

To perform indirect somatic embryogenesis, Eisa et al. (2005) induced callus from one-week-old hypocotyls (0.5–1 cm) on modified MS supplemented with 0.1 mg/L 2,4-D under a 16-hour photoperiod at 25 °C. After four weeks, a colorless callus was obtained with an efficiency of 92%. Subcultures were performed to maintain the calli without regeneration on a solid or liquid medium supplemented with 0.1 mg/L 2,4-D. The four-week-old callus was subcultured either on solid, modified MS medium or liquid, modified MS medium (agitation at 90 rpm) without any supplementation. Both media induced somatic embryogenesis, leading to an embryo-to-seedling conversion rate of 5%. However, they do not report the variety

used, limiting the reproducibility and scope of the results. Sağlam and Doğancı (2018) also induced embryogenic callus on MS supplemented with 0.5 mg/L 2,4-D from apical segments of Limtar Red quinoa seedlings with an efficiency of 100% (Table 3). Although the authors report this protocol as embryogenic, there is no evidence to support this claim, as no somatic embryo induction was performed, nor was histological examination of the callus conducted to confirm the presence of embryogenic structures.

For the reports on somatic embryogenesis, both of them use MS supplemented with 2,4-D to induce callus from shoot tips or hypocotyls with high efficiency (100% and 92%, respectively). As shown by Eisa et al. (2005) in quinoa, switching from a medium with high auxin concentration to a medium with low concentration is usually enough to induce the formation of somatic embryos (Salaün et al. 2021). In this regard, auxins, and especially 2,4-D, are essential for somatic embryogenesis induction (Wójcik et al. 2020). In *Amaranthaceae*, 2,4-D was also reported to induce somatic embryogenesis in *C. murale* and *O. rubra*. (Milivojević et al. 2005), but somatic embryogenesis is not limited to 2,4-D and other growth regulators, and explants can be used, as in spinach (Zdravković-Korać et al. 2023).

Indirect organogenesis in quinoa

The formation of organs by indirect organogenesis requires the initial formation of a callus initiated from different organs, such as hypocotyls, with the help of 2,4-D alone or in combination with another growth regulator (Custódio et al. 2022). In quinoa, only two papers have been published for indirect organogenesis (Fig. 2) (Hesami and Daneshvar 2016; Xie et al. 2025).

Hesami and Daneshvar (2016) induced callus from eight- to 10-day-old hypocotyl segments (0.5–1 cm) on MS medium supplemented with 0.5 mg/L 2,4-D and 0.05 mg/L BAP. The calli were induced in darkness at 25 °C; after four weeks, the calli were weighed. These conditions produced 93% efficiency with an average callus of 1.2 g. The heaviest four-week-old calli were transferred to MS medium supplemented with 1 mg/L BAP, 1 mg/L KIN, and 0.2 mg/L IBA to induce shoot formation. The calli for regeneration were kept under a 16-hour photoperiod at 25 °C. After four weeks, the shoot formation efficiency was 86%, with an average of 6.3 shoots/explant and a length of 2 cm per shoot. However, the researchers do not report a rooting protocol or the variety of quinoa used, which limits the scope of the research and its reproducibility.

Xie et al. (2025) induced callus from 3 to 4 mm hypocotyl segments excised from three- to five-day-old Qingqua I plants on MS supplemented with 30 g/L sucrose, 0.2 mg/L BAP, and 2 mg/L NAA. The cultures were maintained under

a 16-hour photoperiod at 25 °C and subcultured once on the same medium after 3 weeks, resulting in an efficiency of 97% after 42 days. To induce shoot formation, the calli were placed on MS supplemented with 30 g/L sucrose, 1 mg/L BAP, 0.1 mg/L NAA, and 0.2 g/L proline. The cultures were maintained under a 16-hour photoperiod at 25 °C for 56 days, resulting in an efficiency of 89% (40.6 shoots/explant). Clusters of shoots were then placed on 3MS supplemented with 30 g/L sucrose, 0.1 mg/L BAP, and 0.01 mg/L NAA. The cultures were maintained under a 16-hour photoperiod at 25 °C for 35 days, resulting in an efficiency of 88% with an average shoot length of 6.85 cm. Shoots (> 2 cm) were transferred to MS supplemented with 30 g/L sucrose and 0.6 mg/L IBA to induce rooting. The cultures were maintained under a 16-hour photoperiod at 25 °C for 30 days, resulting in an efficiency of 98% with an average of 16.9 roots per explant.

To induce callus, both papers used hypocotyls from 10-day-old (Hesami and Daneshvar 2016) and five-day-old seedlings (Xie et al. 2025) on MS supplemented with BAP combined with 2,4-D (Hesami and Daneshvar 2016) or NAA (Xie et al. 2025). To induce shoots from callus, both protocols used BAP combined with IBA and KIN (Hesami and Daneshvar 2016) or NAA and proline (Xie et al. 2025). After this step, the difference in efficiency between the two protocols is noticeable. While Hesami and Daneshvar (2016) obtained 6.3 shoots per explant, Xie et al. (2025) obtained six times more shoots per explant. Besides, Hesami and Daneshvar (2016) do not report a shoot multiplication step, a rooting step, or the quinoa variety used. Altogether, the lack of this information limits the scope of the research. In *Amarantaceae*, MS supplemented with BAP and 2,4-D was also used to induce callus formation in sugar beet. In this case, callus was induced from cotyledons on MS supplemented with 0.1 mg/L BAP and 2 mg/L 2,4-D. The callus was then transferred to MS supplemented with 1 mg/L BAP and 0.2 mg/L IAA to induce shoot formation (Morsi et al. 2019).

Direct organogenesis in quinoa

Micropropagation, the in vitro multiplication of plants under controlled conditions, is achieved through either somatic embryogenesis or organogenic pathways. Among the organogenic pathways, the direct organogenesis pathways include axillary branching and *de novo* bud formation (Purohit et al. 2011). So far, six papers on direct organogenesis in quinoa have been published (Fig. 2). Two of them report shoot or flower induction without rooting steps (Hesami et al. 2018; Sidorov et al. 2024) and four reports a micropropagation protocol (Burnouf-Radosevich and

Paupardin 1985; Regalado et al. 2020; Al Gethami and El Sayed 2020b; Al Gethami and Sayed 2020a).

Hesami et al. (2018) induced shoots from four-week-old cotyledonary node segments (0.5–1 cm) on MS medium supplemented with 2 mg/L BAP under a 16-hour photoperiod at 25 °C. After four weeks, shoot formation efficiency was 93%, with an average of 4.96 shoots/explant and 4.83 cm of length per shoot. The cultures were kept fresh by monthly subcultures, but no root induction or variety used was reported.

Sidorov et al. (2024) induced flowers from inflorescence obtained from *in vitro* germinated plants. Seedlings developed a highly branched inflorescence one month after germination, which was used to induce flower formation. To induce the culture, chopped inflorescences were cultured on DKW medium supplemented with 20 g/L sucrose, 1 mg/L KIN, 0.5 mg/L BAP, and 0.1 mg/L gibberellic acid (GA3) under a 16-hour photoperiod at 25 °C. The culture was ready for genetic transformation after three to four subcultures (12- to 14-day intervals), but no efficiency was reported. In this case, the explants failed to produce roots; therefore, the authors grafted the induced transformed explants onto wild-type plants.

Burnouf-Radosevich and Paupardin (1985) induced buds from six-day-old shoot tips on a B5 medium supplemented with 0.22 mg/L BAP and 0.018 mg/L NAA. The efficiency of this treatment was 67% with 3.78 buds per explant. Buds were again induced from shoots obtained from the last treatment on a modified B5 medium supplemented with 0.22 mg/L BAP and 0.018 mg/L NAA. The modifications of B5 medium were: 10 g/L sucrose, 2.7 g/L KNO₃, 315 mg/L NaH₂PO₄·H₂O, and 4 mg/L glycine. This treatment produced 8.46 shoots per explant. The cultures were maintained in a growth chamber under a 12-hour photoperiod at 20 °C, with evaluations performed after three weeks and subcultures performed every three weeks. The rooting of explants was performed using shoots propagated for two years on a modified B5 medium. One month later, 60% of the explants developed roots on modified B5 medium without supplementation; however, the rooting rate increased to 75% when the temperature during the day was 20 °C and 4 °C at night.

Regalado et al. (2020) induced shoot formation from seven-day-old apical segments (3 mm) on MS medium supplemented with 1 mg/L KIN and 1 mg/L BAP. The efficiency of this treatment was 93% (Cahuil) and 79% (Villa), with 2.14 shoots per explant in Cahuil and 1.43 in Villa after four weeks of culture. A shoot multiplication step was performed using excised shoots from the induction step, on MS medium supplemented with 2 mg/L BAP and solidified with 8 g/L bacteriological agar. The cultures were evaluated four weeks later, producing 7.96 shoots per explant for Cahuil

and 4.1 for Villa. 1.5–2 cm-long shoots were subcultured on MS medium supplemented with 2 mg/L IBA solidified with 8 g/L bacteriological agar, with rooting rates of 87% (Cahuil) and 83% (Villa), with acclimatization success of 83% (Cahuil) and 80% (Villa). The cultures were maintained under a 16-h photoperiod at 25 °C.

Al Gethami and El Sayed (2020a) induced bud formation from 15-day-old cotyledonary nodes (0.5–1 cm) from Faro quinoa plants on MS medium supplemented with 10 g/L sucrose. After four weeks of culture, 93% of the explants responded to the treatment, producing an average of four shoots per explant, with an average length of 2.33 cm per shoot. Shoots previously induced were transferred to a half-strength MS medium supplemented with 10 mg/L ZnO-NPs to induce rooting. After four weeks of culture, 100% of shoots developed an average of 4.8 roots per shoot. The cultures were kept in a growth chamber under a 12-h photoperiod at 25 °C. Using similar conditions Al Gethami and El Sayed (2020b) induced shoots from cotyledonary nodes on MS supplemented with 10 g/L sucrose. After four weeks of culture, 93% of the explants responded to the treatment, producing an average of four shoots per explant. The obtained shoots were cultivated on half-strength MS medium to induce roots with an efficiency of 60%, yielding 0.8 roots per shoot. However, the researchers do not report the variety of quinoa used, which limits the scope of the research and its reproducibility.

The direct organogenesis reports in quinoa are also heterogeneous in the growth regulators used to induce shoot or flower formation from meristem-rich explants. BAP, NAA, KIN, and GA3 have been used to induce shoots from shoot tips (Burnouf-Radosevich and Paupardin 1985; Regalado et al. 2020), cotyledonary nodes (Hesami et al. 2018; Al Gethami and El Sayed 2020b; Al Gethami and Sayed 2020a), and flowers from quinoa inflorescences (Sidorov et al. 2024). The organ induction is mainly performed on MS, except for Burnouf-Radosevich and Paupardin (1985) who used B5 (with an efficiency of 67%, the lowest so far) and Sidorov et al. (2024), who used DKW, but did not report an efficiency. Finally, most of the researchers reported the variety used, with two exceptions (Hesami et al. 2018; Al Gethami and El Sayed 2020b).

Only Burnouf-Radosevich and Paupardin (1985) and Regalado et al. (2020) reported a shoot multiplication step using BAP alone (Regalado et al. 2020) or combined with NAA (Burnouf-Radosevich and Paupardin 1985), with efficiencies of 4.1 shoots per explant for Villa variety and 7.96 shoots per explant for Cahuil variety (Regalado et al. 2020) and 8.46 shoots/culture for Blanca de Junin (Burnouf-Radosevich and Paupardin 1985). Surprisingly, only Regalado et al. (2020) used IBA to induce root formation in Villa and Cahuil varieties. The other papers used ZnO-NPs

(Al Gethami and El Sayed 2020a) or no supplementation at all (Burnouf-Radosevich and Paupardin 1985; Al Gethami and El Sayed 2020b). The lowest rooting efficiency (60%, 0.8 roots/explant) was obtained on MS without supplementation (Al Gethami and El Sayed 2020b), while the highest (100%, 4.8 roots/explant) was obtained on MS supplemented with ZnO-NPs (Al Gethami and El Sayed 2020a), showing that nanoparticles could have a positive effect on the rooting step.

As shown in quinoa by Hesami et al. (2018), shoot buds were induced from internodal segments in *Alternanthera sessilis*, another member of the Amaranthaceae family, also on MS supplemented with BAP (Sowmya et al. 2020). Besides, the regeneration capacity of inflorescences and flowers is not exclusive to quinoa (Sidorov et al. 2024) and has been demonstrated in other species of the Amaranthaceae family. In *Atriplex glauca*, shoots were induced from flowers using 0.1 mg/L 6-(γ - γ -Dimethylallylamino)-Purine (2iP) and 0.01 mg/L IAA (Kenny and Caligari 1996). In sugar beet, bud clumps were induced from immature inflorescence on MS supplemented with 1 mg/L BAP and 0.25 mg/L NAA (Yang et al. 2004).

Within the Amaranthaceae family, micropropagation protocols through direct organogenesis pathways have been published. Shoots were induced from spinach petioles on MS supplemented with 1 mg/L BAP, 0.4 mg/L NAA, and 2 mg/L GA3. For rooting, the regenerated shoots were submerged in 20 mg/L IBA for 2 h and then transferred to MS supplemented with 0.025 mg/L IBA (Hussain et al. 2024). For *A. reineckii*, shoots were induced from nodal segments on MS supplemented with 1 mg/L BAP and illumination of one part red and two parts blue light. A piece of the regenerated shoot was placed on MS supplemented with 0.5 mg/L IAA to induce root formation (Ekin and Dogan 2024).

Genetic transformation protocols in quinoa

The use of biotechnological methods has helped to improve agronomic properties in plants, with genetic engineering enhancing valuable properties and introducing new characteristics to crops through *Agrobacterium*-mediated gene delivery and direct delivery using biolistic (Anuradha et al. 2023). Although *Agrobacterium*-mediated transient genetic transformation is the most common method in quinoa, a few *Agrobacterium*-mediated stable genetic transformation methods have been reported (Fig. 1), as summarized in Table 4.

A. tumefaciens-mediated transient genetic transformation

The transient transformation methods are routinely used, usually through agroinfiltration of leaves, enabling a fast and straightforward method for functional screens (Krenek et al. 2015). This method also enables transient expression of the CRISPR-Cas9 machinery, producing gene edits without the creation of a transgenic intermediate (Nekrasov et al. 2013). In quinoa, two papers successfully reported an agroinfiltration protocol (Xiao et al. 2022; Wang et al. 2023).

Xiao et al. (2022) transiently transformed *A. tumefaciens* GV3101 leaves of NL-6 and Ames13752 quinoa varieties. The bacteria were resuspended in infiltration buffer (0.25 M MS, 10 mM MgCl₂, 200 μ M AS, 1% sucrose, 0.01% Silwet L-77, adjusted to pH 6) to an OD₆₀₀ of 0.5 and injected with a syringe (without needle) into the leaf or axillae. The plants were kept in the dark overnight and then moved to the greenhouse for two days before sampling. The method's effectiveness was tested by measuring the gene expression of heterologous genes or by detecting gene editing events. With the protocol described above, the authors successfully edited the genes *CqPHR1* and *CqTSARL3* using CRISPR-Cas9, demonstrating that gene editing is possible in quinoa. Although no stable edited plants were achieved, the method could be used to test which promoters and constructs work best in quinoa cells.

Wang et al. (2023) established an agroinfiltration protocol for transient transformation using two-week-old quinoa leaves (cultivar MS509/N). *A. tumefaciens* EHA105 was grown for two days on plates with YEP medium, regrown for another day on YEP medium to finally collect the cells and resuspend them with infection medium (0.1 M MS, 19.6 mg/L AS, 100 mg/L MES and 2% sucrose, adjusted to pH 5.6) to an OD₆₀₀ of 1. The bacterial culture was injected with a syringe (without a needle), and the injected seedlings were covered and grown under a 16-hour photoperiod at 22 °C for 4 days. The leaves were then GUS-stained (Jefferson et al. 1987) to detect β -glucuronidase activity, but no efficiency was reported, limiting the reproducibility of the research.

Both agroinfiltration protocols used leaves as targets, with strain GV3101 carrying pCambia-based vectors at an optical density (OD₆₀₀) of 0.5 (Xiao et al. 2022) and EHA105 carrying pBI121 at an OD₆₀₀ of 1 (Wang et al. 2023). Xiao et al. (2022) reported a transformation efficiency of 25% after overnight incubation; however, no efficiency was noted for edits. Similarly, Wang et al. (2023) do not report transformation efficiency. In quinoa, the agroinfiltration method has also been used with virus-induced gene silencing (VIGS) of *CqERF24* (Zhu et al. 2023), phytoene desaturase (*CqPDS*) (Melgar et al. 2024), *CqBIN2* (Zhou et al. 2024), and overexpression of auxin-related genes (Sun et

Table 4 Genetic transformation of quinoa: varieties, explants, and methods used in published protocols

Variety	Explant	Method	OD ₆₀₀	Infection time	Coculture/ Incubation (days)	Vector	Selection/Marker	Efficiency	Limitation	Reference
Not indicated NL-6, Ames13752	Cellular suspensions	<i>A. tumefaciens</i> A281	0.8	5 min	2	pTOK162	Kanamycin	10.8 calli per plate	No regeneration reported	Komari (1990)
	Leaves	Agroinfiltration (<i>A. tumefaciens</i> GV3101)	0.5	Not performed	Overnight, dark conditions	pCAMBIA1300-based	GUS staining	Transformation: 25% Editing: not indicated	Transient expression	Xiao et al. (2022)
MS509/N	Cotyledon node	<i>A. rhizogenes</i> K599	1	30	2, dark conditions	pTF102	GUS staining	67.9%	No regeneration reported	Wang et al. (2023)
	Cotyledon		1	30	2, dark conditions	pTF102	GUS staining	42.4%	No regeneration reported	
	Hypocotyl		1	30	2, dark conditions	pTF102	GUS staining	31.6%	No regeneration reported	
	Leaves	Agroinfiltration (<i>A. tumefaciens</i> EHA105)	1	Not performed	4, photoperiod (16-hour/ 8 h)	pBI121	GUS staining	Not indicated	Transient expression	
QQ74	Flower-derived culture	<i>A. tumefaciens</i> AB32	0.8-1 (OD ₆₀₀)	5 min in vacuum infiltration plus 30 min centrifugation	4-5, photoperiod (16-hour/ 8 h)	pVSI-based	Paromomycin/GUS staining	20%	Long time frame	Sidorov et al. (2024)
Real	Inflorescence	<i>Agrobacterium</i> -mediated floral dip	Not indicated	Not indicated	Not indicated	pHSE401	Transformants (T0) identified by PCR	Transformation: 18.7% Editing: 41.7-75%	Absence of a detailed protocol	Bu et al. (2025)

al. 2022) and *Cqtrihelix23* (Sun et al. 2024). In *A. tricolor* and spinach, an agroinfiltration method was used to express isoflavone synthase from soybeans, resulting in the transient production of genistein (Malla et al. 2021).

***Agrobacterium*-mediated stable genetic transformation**

For stable transformation, the integration of foreign DNA into the host plant's genome is necessary to pass it on to the offspring, for example, to enhance stress resistance and produce biomaterials and secondary metabolites in transformed plants. The vast majority of the transgenics have been generated using *A. tumefaciens* (Azizi-Dargahlou and Pouresmaeil 2024). In quinoa, three papers successfully reported in vitro culture-dependent stable transformation protocols with *A. tumefaciens* (Komari 1990; Sidorov et al. 2024) and *A. rhizogenes* (Wang et al. 2023).

From leaf-derived calli, Komari (1990) initiated suspension cultures on liquid LS medium supplemented with 3 mg/L IAA, 3 mg/L NAA, and 0.1 mg/L KIN, with weekly subcultures. Around 1×10^4 cell clusters (10–30 cells) were suspended in 2 mL medium and incubated with 1×10^8 *A. tumefaciens* A281 (pTOK162) cells for 48 h. Later, the bacteria were washed from the quinoa cells, and the cells were spread on a medium supplemented with 250 mg/L cefotaxime and 300 mg/L kanamycin (Komari 1989). After six weeks of selection, the cultures were evaluated, obtaining an average transformation efficiency of 10.8 kanamycin-resistant calli per plate. However, the researchers do not report the regeneration of plants nor the variety used, which limits the applicability of the protocol for obtaining transformed plants.

Sidorov et al. (2024) established an *Agrobacterium*-mediated transformation protocol using flower-derived culture. The culture was ready for transformation a few subcultures later (intervals of 12 to 14 days) on the induction medium. 500 µL of *Agrobacterium tumefaciens* strain AB32 was grown in 200 mL of LB medium for 20–22 h at 28 °C and 200 rpm until the optical density at 660 nm (OD_{660}) reached 1.2–1.4. The bacterial culture was centrifuged and resuspended to an OD_{660} of 0.8–1.0 in 0.5 MS (with vitamins) supplemented with 68.5 g/L sucrose, 36 g/L glucose, 0.115 g/L L-proline, and 200 µM acetosyringone (AS). Approximately 200 explants (weighing around 140 g) were vacuum-infiltrated for 5 min with 150 mL of bacterial culture in 250 mL Corning bottles. The cultures were centrifuged (320 g, 30 min), sieved, and dried on sterile paper for 3–5 min. A sterile Whatman filter paper was moistened with the inoculation medium, and the dried cultures were spread on it. The plate was then incubated for four to five days under a 16-hour photoperiod at 65% relative humidity

and 21 °C. After coculture, the explants were transferred to DKW medium supplemented with 20 g/L sucrose, 1 mg/L KIN, 0.5 mg/L BAP, 0.1 mg/L GA3, 50 mg/L paromomycin, 750 mg/L carbenicillin, and 300 mg/L cefotaxime under a 16-hour photoperiod at 25 °C, with subcultures every 12 to 14 days, reducing carbenicillin (500 mg/L) and cefotaxime (200 mg/L). Explants were transferred to DKW medium without growth regulators after three to three and a half months to induce the formation of flowers and shoots. The transformed explants were evaluated by growth under selection and GUS staining (Jefferson et al. 1987), yielding a 20% efficiency (transformed events per initial explant).

In general terms, the protocol is lengthy, and the absence of a rooting step could challenge the obtaining of seeds from the transformed flowering shoots. For this reason, explants were grafted onto wild-type quinoa seedlings. The idea behind this method was to increase the seed number; however, grafting opens up a previously unexplored method in quinoa: grafting wild-type scions onto transgenic rootstock expressing root-to-shoot mobile RNA to produce, for example, transgene-free edited plants (Yang et al. 2023).

Wang et al. (2023) established a genetic transformation protocol using *A. rhizogenes* for quinoa. Hypocotyl, cotyledon, and cotyledon nodes of five- to six-day-old plants were used as explants. The cotyledon nodes and cotyledon explants were pierced with a needle a few times before transformation. *A. rhizogenes* K599 transformed with the vector pTF102 was grown for two days on plates with YEP medium, regrown for another day on YEP medium to finally collect the cells, and resuspended with infection medium (MS, 19.6 mg/L AS, 500 mg/L MES, and 3% sucrose, adjusted to pH 5.4) to an optical density of 1. Explants were incubated with the bacteria in the infection medium for 30 min at 25 °C and then transferred to a coculture medium for two days in darkness at 22 °C. Explants were incubated in darkness on a hairy-root-induction medium (B5, 500 mg/L MES, and 3% sucrose, adjusted to pH 5.6) at 25 °C for two weeks to induce hairy root formation. Explants were then GUS-stained to evaluate transformation efficiency, yielding the following results: cotyledon nodes, 67.9%; cotyledons, 42.4%; and hypocotyls, 31.6%. Once again, the researchers do not report plant regeneration from the explants used, limiting the applicability of the method. Although a protocol for regenerating shoots from roots has not yet been reported in quinoa, plant regeneration after *A. rhizogenes*-mediated transformation is possible in Amaranthaceae (Castellanos-Arévalo et al. 2021).

To achieve stable transformation of quinoa, either using *A. rhizogenes* (Wang et al. 2023) or *A. tumefaciens* (Komari 1990; Sidorov et al. 2024) in an in vitro culture-dependent manner, researchers used similar optical densities for the bacteria, ranging from 0.8 to 1. All the papers

reported the efficiency calculated by GUS-staining, but only two reported the variety used, being MS509/N (Wang et al. 2023) and QQ74 (Sidorov et al. 2024), affecting the reproducibility of the one with incomplete information (Komari 1990). The highest efficiency was obtained in cotyledon nodes transformed with *A. rhizogenes*; this transformation protocol could benefit from an in vitro culture-dependent or an in vitro culture-independent method (Cao et al. 2023) to regenerate shoots from roots.

Within the Amaranthaceae family, hygromycin-resistant calli were obtained from transformed hypocotyls of waterhemp. First, the hypocotyls were exposed to *A. tumefaciens* EHA105 ($OD_{600}=0.8$) for 5 min and then placed on a callus-induction medium supplemented with 15 mg/L hygromycin for selection. The hygromycin-resistant calli were evaluated by PCR, yielding a transformation efficiency of 1.5% (Kumam et al. 2024). Although no protocols transforming flower cultures (Sidorov et al. 2024) has been reported for other species of the Amaranthaceae family, immature inflorescences from sorghum were chopped and transformed with *A. tumefaciens* LBA4404. Afterward, shoots were obtained from the embryogenic callus induced after transformation (Chou et al. 2020). Transformation of other types of explants is also possible in the Amaranthaceae family. Hand-pricked epicotyls from *A. tricolor* were transformed with *A. tumefaciens* EHA105 ($OD_{600}=0.6$). After infection with AS-treated bacteria for 10 min, the explants were cocultured for four days on regeneration medium (Pal et al. 2013). In sugar beet, shoots and petioles were vortexed with *A. tumefaciens* LBA4404 and silver sand for 30 min, cocultured for three days, transferred to a growing medium for four weeks, and then moved to a selection medium (Gurel 2021).

The induction of hairy roots by *A. rhizogenes* has also been utilized in various species of the Amaranthaceae family. In sugar beet, the petiole and midrib were submerged in an *A. rhizogenes* A4T or LBA1334 culture ($OD_{600}=0.5$), sonicated for 15 s, and then shaken for 15 min. Explants were cocultured for three days in the dark, with 54% of the explants developing hairy roots (Klimek-Chodacka and Baranski 2014). Cotyledons of *A. hypochondriacus* and *A. hybridus* were exposed to *A. rhizogenes* BVG ($OD_{600}=0.4$ and 0.8, respectively). Hairy roots were validated by PCR, Green Fluorescent Protein (GFP) emission, and GUS staining for both species. Interestingly, somatic embryogenesis was induced from the hairy roots of *A. hypochondriacus* (Castellanos-Arévalo et al. 2020).

Tissue-culture-independent transformation methods in quinoa

Genetic transformation tissue culture methods are laborious and time-consuming, as they require optimization at all stages (Sebiani-Calvo et al. 2024). Therefore, tissue-culture-independent transformation protocols could overcome the limitations of tissue culture (Sebiani-Calvo et al. 2024), including somaclonal variations (Castellanos-Arévalo et al. 2021). The floral dip method, initially developed for *Arabidopsis* (Clough and Bent 1998), remains challenging to apply in the Amaranthaceae family, given the morphological and physiological differences between them and *Arabidopsis*; however, the floral dip method has already been used in quinoa (Bu et al. 2025).

Bu et al. (2025) transformed Real quinoa plants with the *Agrobacterium*-mediated floral dip method. Inflorescences at the early flowering stage were dipped into *A. tumefaciens* transformed with vector pHSE401. Although the authors report a transformation efficiency of 18.7% as evaluated by PCR, they do not provide details on the methods, which limits the reproducibility of the technique. The main objective of this research was to increase the nutritional value of quinoa seeds by multiplexed CRISPR-mediated mutation of *CqAAP1*, *CqIPK1*, *CqGGP*, and *CqHPT*. The authors argue that the editing efficiency ranges from 41.7% to 75%, and that higher concentrations of lysine, zinc, vitamin C, and E are found in edited seeds. Although this is not the first report using CRISPR-Cas9 in stably transformed tissue to mutate a member of the Amaranthaceae family (Vollmer et al. 2025), it is the first one in quinoa.

Within Amaranthaceae, *Amaranthus* (Munusamy et al. 2013; Yaroshko et al. 2018, 2020) and *O. rubra* (Veit et al. 2006) plants have been transformed with the floral dip method. Although in *O. rubra* the transformation by floral dip was proven to be a false positive (Drabešová et al. 2016), the method remains a good alternative to transformation methods that include in vitro culture steps (Xiao et al. 2022). Another tissue-culture-independent transformation method applied to *O. rubra* was the sonication-assisted *Agrobacterium*-mediated transformation (SAAT) (Flores Solís et al. 2003). In this method, young seedlings are exposed to *Agrobacterium* under sonication, producing micro-wounds in which the bacteria can infect the cells (Flores Solís et al. 2003), potentially transforming meristematic tissue (Trick and Finer 1997). Although longer sonication times could damage the tissue and reduce responses of the treated tissue to in vitro culture (Trick and Finer 1997) it may be necessary to use longer sonication times to apply this method successfully to quinoa, as well as a pretreatment of the quinoa seeds, such as reducing saponin content and softening the seed coat. This method was also used in

sugar beet to induce hairy roots with *A. rhizogenes* (Klimek-Chodacka and Baranski 2014). Although the researchers did not obtain transformed plants, the SAAT method could be worth trying in quinoa, especially in combination with different techniques like Cut-dip-budding (Cao et al. 2023) to produce transformed buds from transformed roots.

The *de novo* induction of meristems by overexpressing meristem-related genes through direct delivery or the Fast-TrACC method may help overcome the limitations of tissue culture (Maher et al. 2020; Nasti et al. 2020; Cody et al. 2022). For both methods, meristem-related genes (for example, *Wuschel* (*Wus*), *Shoot Meristemless* (*STM*), *BABY BOOM* (*BBM*), and isopentenyl transferase (*ipt*) are over-expressed to produce the transformed *de novo* meristems. Although this method has not been used in species of the Amaranthaceae family, it is worth trying it in quinoa. The challenge for this method is determining which gene or combination of genes triggers *de novo* meristem induction. Xiao et al. (2022) have demonstrated that quinoa's axillae can be transiently transformed, rendering the *de novo* induction of meristems feasible.

Concluding remarks and future perspectives

Significant advances have been made in quinoa tissue culture (Fig. 1). Most seed surface sterilization protocols for quinoa include ethanol and sodium hypochlorite, providing a suitable sterilization level for in vitro culture. The most extended in vitro germination method uses full MS with incubation under a 16-hour photoperiod at 25 °C, usually reaching high germination rates. Direct organogenesis from meristem-rich explants is reproducible, and indirect organogenesis from hypocotyl-derived callus supplemented with proline significantly enhances shoot multiplication (Xie et al. 2025). In contrast, convincing evidence for somatic embryogenesis remains scarce since there is no verification that Sağlam and Doğanç (2018) callus is embryogenic. In addition, two stable genetic transformation systems have been reported: *Agrobacterium tumefaciens*-mediated floral culture and floral dip, demonstrating that quinoa is amenable to stable transformation, even though protocols require further optimization.

For future directions, the hypocotyl-based indirect protocol with proline is the most promising baseline to validate across multiple genotypes. Besides, the callus induced by Xie et al. (2025) appears as an interesting target for genetic transformation. In the meantime, the flower culture method currently represents the only stable system for quinoa (Sidorov et al. 2024), albeit with lengthy steps and limitations in rooting of transformed flowering shoots. Ideally, this protocol should be shortened by reducing, for example,

the number of subcultures before transformation. The transformed flowering shoots should then be allowed to develop roots, thereby establishing an in vitro culture-dependent routine transformation method that can be optimized for other varieties. The floral dip, which is the most interesting in vitro culture-independent transformation method, seems somewhat elusive since no detailed method has been published (Bu et al. 2025). Related to this, reporting remains incomplete in many studies (cultivar identity, light intensity in PPFD, operational definition of “efficiency”), which prevents reproducibility and meta-analysis.

In this sense, to improve reproducibility and cross-study comparisons, we recommend that future reports explicitly include variety/cultivar, explant age, medium composition (salts and vitamins), growth regulators, gelling agent, light intensity (preferably in $\mu\text{mol m}^{-2} \text{s}^{-1}$), culture vessel and subculture interval, operational definition of “efficiency,” and details of *Agrobacterium* inoculation (strain, OD, coculture) and selection regime. Having this information systematically reported will enable more informed decisions regarding the future development of in vitro culture and genetic transformation protocols in quinoa. In particular, standardized datasets would facilitate the application of machine learning and other data-driven approaches to identify key factors, optimize conditions, and accelerate the development of protocols. Altogether, the systematic generation and sharing of such knowledge will not only advance quinoa tissue culture and transformation research but also strengthen breeding programs and contribute to securing future global food availability.

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Declarations

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