Weed Technology

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Weed seed detection in plant quarantine: Practices and emerging molecular technologies

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Review

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Abstract

Over the years, the rapid and widespread spread of weeds across international borders has become a major threat to agriculture and native ecosystems. Seeds of invasive species are often transported via commodity trade, making rapid and accurate identification essential. This review aims to explore current practices at plant quarantine centers worldwide for detecting weed seeds and to examine potential molecular techniques that could revolutionize this process in the future. Book chapters, scholarly research articles, conference proceedings, and previously published literature on weed seed detection approaches and plant quarantine inspections in various countries were reviewed, examined, and compared to identify the tools and techniques currently used or potentially developed. The literature survey revealed that countries employ diverse strategies for issuing phytosanitary certificates for import and export commodities, adhering to agreements established by global and regional plant protection organizations. Both traditional morphology-based techniques and advanced molecular tools were identified in these practices. Weed seeds present particular detection challenges at quarantine ports, including physiological dormancy that undermines germination-based tests, morphological similarity to crop seeds, and zero-tolerance phytosanitary thresholds for certain noxious species. The review identified various deviations and advanced methods for detecting weed seeds in import and export commodities, aiming to determine the most appropriate or suitable technique for plant quarantine services in worldwide.

Introduction

Weeds are defined as plants that spontaneously grow on human-modified lands (Godinho 1984) while fertile weeds are those specifically occurring in frequently cultivated fields. Some weeds have the potential to become invasive in novel environments because they possess competitive characteristics, including rapid reproduction (van Kleunen et al. 2016), fast growth (Sardana et al. 2017), high phenotype plasticity (van Kleunen et al. 2016), high dispersibility (van Kleunen et al. 2016), and high adaptability (Pilu et al. 2012). Weeds can adversely affect agricultural systems by reducing crop yield and land value. Some of these weeds can carry pathogenic microorganisms, insect pests, parasites, and other threatening biological agents (Mack et al. 2000; McNeely 2001). The hybridization of weeds with closely related plants can pose serious threats to native plant populations and agriculture (Ejeta and Grenier 2005), potentially leading to ecological changes and/or financial losses (Panzavolta et al. 2021).

Some weeds are spreading uncontrollably across the globe, often through unintentional human-mediated movement from one region to another. International commerce is the primary cause of such weed invasions, enabling plants to spread beyond their native ranges (Pyšek et al. 2020). While natural dispersal can occur, human-mediated movement occurs on a much larger scale. These weeds can be transported in the form of foreign plant materials, contaminants in commercial seed lots, or attached to animal furs, human clothing, machines, or bags (Rubenstein et al. 2021).

Weed seeds possess several biological characteristics that make them more challenging to detect at ports of entry than other plant quarantine pests. Some weed seeds such as wild oats (*Avena fatua* L.) and johnsongrass [*Sorghum halepense* (L.) Pers.] show physiological dormancy, which pauses seed germination for an extended period. Such seeds remain dormant in germination-based detection tests, causing false-negative results. Weed seeds are frequently small. Small seeds can easily pass through standard sieves used in mechanical separation of commodity lots. Another feature is that seeds of quarantine weeds are morphologically similar to crop seeds. For example, barnyardgrass [*Echinochloa crus-galli* (L.) P. Beauv.] seeds are highly similar to certain rice varieties and little canary grass (*Phalaris minor* Retz.) is a common contaminant in wheat shipments. Morphologically similar weed species are difficult to detect through visual inspection. Phytosanitary regulations for many noxious weeds have zero-tolerance thresholds, meaning that not even a single regulated seed is allowed in a consignment

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such as purple witchweed [*Striga hermonthica* (Del.) Benth.] under International Plant Protection Convention (IPPC) standards. Collectively, these factors necessitate the development of detection methods that are highly sensitive, species-specific, and able to perform reliably even on small, morphologically indistinct, or physiologically dormant seeds.

There are several notable examples of species that have become worldwide weeds through contaminated seed movement. Ragweed (*Parthenium hysterophorus* L.) was introduced to Asia, including Sri Lanka and India, during the 1950s through contaminated cereal and grass seed shipments from the United States, and subsequently became a highly invasive weed affecting rangelands and agriculture (Bhowmik and Sarkar 2005). In Australia, johnsongrass was introduced through contaminated crop seeds and has since emerged as a costly pest in cereal and cotton fields (Peerzada et al. 2023). New Zealand's border inspections have occasionally detected quarantine weeds, including johnsongrass and goosefoot (*Chenopodium* spp.), in imported seed lots, highlighting how easily unwanted plants can spread through international trade (Rubenstein et al. 2021). In Kenya, mesquite [*Neltuma juliflora* (Sw.) Raf.] spread after its seeds were unintentionally mixed with pasture seed shipments, leading to farmland degradation and the displacement of native vegetation (Mungoche et al. 2025). These examples demonstrate that weed seed contamination can have long-term agricultural, ecological, and economic impacts, underscoring the critical need for stringent quarantine and inspection practices to prevent new invasions.

Rapid and accurate identification of weed seeds present as contaminants is crucial for ensuring the financial and ecological security of ecosystems and agriculture (Ismail et al. 2016). Rapid seed identification strategies can be applied at multiple stages, including seed production, seed grading, and quarantine procedures for imports and exports, and species improvement research (ElMasry et al. 2019; Luo et al. 2023). Further, early detection of weed seeds at exchange centers in every country is necessary to prevent the export and import of foreign plant materials (Reichard 1997). Given the significant potential for alien weeds to spread due to contaminated seed lots (Montagnani et al. 2022), this review focuses on evaluating and comparing techniques for the rapid and accurate detection of weed seeds in plant quarantine settings. By linking weed seed biology with detection constraints and operational realities, we reframe weed seed identification as a systems-level challenge rather than solely a technical problem.

Worldwide Organizations that Govern Plant Quarantine Inspections

Plant quarantine is a legal constraint imposed on the movement of agricultural commodities to exclude, prevent, or stifle the spread of plant pests and diseases in uninfected regions (Chao 2008). The primary aim of plant quarantine is to prevent the unintentional introduction of exotic pests, weeds, and pathogens that could harm a nation's agriculture and environment, and to stop their establishment and subsequent distribution if introduced (Henson and Loader 2001). Authorized organizations worldwide, known as plant quarantine service centers, conduct inspections of quarantine species at airports, seaports, and land borders, implementing plant quarantine regulations (Ambe 2015; Chand et al. 2017).

The IPPC, founded in 1951, and now operating in 184 member countries, serves as the pivotal global agreement for protecting plant resources against the entry and spread of alien pests while facilitating safe international trade (Stubbings 2005). The most recent revision aligns with the World Trade Organization's Agreement on the Application of Sanitary and Phytosanitary Measures (FAO 1997). The phytosanitary requirements set by the IPPC are supported in the most scientific studies, providing crucial resources to promote the implementation of technically sound global trade standards (Griffin 2018).

Regional plant protection organizations (RPPOs) assist their members in achieving the IPPC's goals by supporting initiatives to detect pests, disseminate information, and apply global standards for quarantine measures (APPPC 2005; Ebbels 2003; Petter et al. 2014). One such organization is the North American Plant Protection Organization (NAPPO), established jointly by the United States of America, Canada, and Mexico in 1976 (Ebbels 2003). Nine additional RPPOs operate worldwide (Bloem 2016). These are the Asia and Pacific Plant Protection Commission (APPPC 2005), Caribbean Agricultural Health and Food Safety Agency (Anderson 2010), Andean Community, Southern Cone Plant Health Committee, European and Mediterranean Plant Protection Organization (EPPO 1997), International Regional Organization for Agricultural Health, Near East Plant Protection Organization, Inter-African Phytosanitary Council (Olembo 2003), and Pacific Plant Protection Organization (Ikin 2002). Together, these organizations form a global network that coordinates phytosanitary standards and supports member nations in preventing the spread of invasive plant species through trade.

In Sri Lanka, the National Plant Quarantine Service (NPQS) was established in 1994 as the country's legal defense system to control the movements of invasive alien weeds (IAWs). The NPQS is responsible for preventing the entry of IAWs into the country or identifying them early in export-ready and imported commodities, thus ensuring compliance with national agricultural and environmental policies (Ministry of Agriculture and Agrarian Services n.d.).

It is difficult to detect weeds early because the current procedures are time-consuming and often unreliable. For example, certain tests, such as grow-out tests, require a minimum of 28 d to obtain results. Other tests may yield false negative results due to the dormancy of seeds or the presence of weeds that are morphologically similar to certain crop species. Furthermore, lengthy screening tests may cause significant economic losses for importers and exporters as goods are detained at ports prior to release, this also creating frustration among commercial agents (Wickramaarachchi, NPQS personal communication). Therefore, an urgent need exists to adapt techniques that can rapidly and reliably identify IAWs at quarantine facilities. The following sections review the various methods for weed seed detection currently used or under development by plant quarantine agencies worldwide.

Detection Techniques for Quarantine Inspections and Future Trends

A variety of detection techniques are employed at quarantine centers that use morphological, physiological, biochemical, and molecular analyses. More specifically these techniques include visual examinations (Agarwal et al. 1998; Dubey and Gupta 2016; Lutz et al. 2010; Thakur et al. 2010; Whattam et al. 2013), sieving and gravity separation techniques (Pavithra et al. 2021), germination tests for soil samples (James et al. 2014), and molecular diagnostic tests (Bhardwaj and Hallan 2019). These methods are used globally for detecting seed and adulterant detection (Huang et al. 2015; Lei et al. 2017; Wahab et al. 2022; Whitehurst et al. 2020; Xiong et al. 2018), with morphological and molecular-based tools

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frequently involved. The choice of method depends on the resources available at a quarantine facility, the urgency of clearance, and the taxonomic complexity of the species being inspected. Each approach has distinct advantages and limitations (Table 1) and is discussed in detail below.

Morphology-Based Approaches

Phenotypic characteristics are often used for recognizing and categorizing seeds (ElMasry et al. 2019; Luo et al. 2023). Specific characteristics include seed size, shape, color, texture, and spectral reflectance. Rapid seed identification has been further advanced through automated image analysis algorithms. These characteristics are nondestructively obtained via machine vision, spectroscopy, and hyperspectral imaging systems (Ahmad et al. 2020; Xu et al. 2022).

Machine vision is a computer-based imaging technique that automatically acquires and analyzes visual information of objects. Spectroscopy can be used to identify seeds by analyzing their unique morphological and chemical characteristics using light. Near-infrared (NIR) spectroscopy and hyperspectral imaging (HSI) are key spectroscopic techniques readily used for identifying different seed types (Wu et al. 2019; Zhang et al. 2018). In NIR spectroscopy, characteristic absorption bands corresponding to specific molecular bonds (e.g., C–H, O–H, N–H) are given. The generated absorption profile provides a biochemical fingerprint of seeds that is ideal for identifying different species or varieties (Zhou et al. 2024).

The HSI technique uses a sophisticated sensing approach that concurrently acquires both spatial and spectral data, facilitating nondestructive and reagent-free analysis of material, including both chemical and biological characteristics (Hong et al. 2026). HSI significantly improves test accuracy compared to simple spectral characteristics alone because it combines seed morphological characters such as details of seed shape and texture (Huang et al. 2016). In recent years, the method has been widely used for seed quality and safety inspection including crop variety detection, viability and vigor determination, and damage detection. The effectiveness of HSI has been significantly improved by combining machine learning and deep learning techniques (Qi et al. 2025). Several recent studies (e.g., Fu et al. 2022, Huang et al. 2024, Li et al. 2024, and Zhang et al. 2023) have used HSI combined with machine learning techniques to improve accuracy of crop variety recognition. For quarantine applications, these systems can theoretically screen large seed lots rapidly; however, their deployment requires substantial upfront investment in specialized equipment and the development of validated reference spectral libraries for target weed species.

Seed lots and other plant-based commodities, especially planting materials, are tested for contamination with weed seeds using seed germination assays in most import and export inspection centers throughout the world. This analysis takes a considerable amount of time, approximately 21 d or more, compared to other techniques. It is primarily used to check the presence of any seed within a consignment to decide on approval or rejection without identifying the specific type of seed (Wilson et al. 2016). In some cases, seedling characteristics are used for morphological identification of weeds, but this process is also time-consuming (Stucky 1984). When using seedling features for identification, their similar and complex morphologies can create obstacles to making accurate plant descriptors (Le et al. 2020). Image texture analysis is one of the techniques that can be used for this purpose (Pietikäinen et al. 2011). However, it faces several challenges, including noise sensitivity and fluctuation in the gray-scale, lighting, and brightness settings (Le et al. 2020).

Local binary patterns (LBPs) represent a computationally efficient method that addresses many limitations (Guo et al. 2010). It is a powerful method for characterizing relationships among pixels in plant images and for detecting microstructure features such as grooves, patches, margins, and flat surfaces (Ojala et al. 2002). This encoding is particularly sensitive to monotonic changes in illumination, making it well-suited for field or field-adjacent inspection locations where lighting conditions may vary. Several variants of the LBP process have been developed to mitigate some of its inherent limitations, and those can be implemented for plant identification. Extended LBP (ELBP) is a texture descriptor that encodes local intensity patterns around a pixel. It is often applied band-wise or on derived spectral features to extract spatial texture information, which is then combined with spectral features to improve classification accuracy (Liu et al. 2012). Noise-tolerant LBP (NTLBP) is a method that minimizes the susceptibility of standard LBP to image noise. Conventional LBP and ELBP are unable to effectively utilize information conveyed by non-uniform patterns and have inherent sensitivity to image noise. To address those limitations, NTLBP extracts both statistical and structural image features, enabling more robust and efficient texture analysis (Fathi and Naghsh-Nilchi 2012). Completed LBP (CLBP) is designed to mitigate the negative effects of conventional LBP by providing a more comprehensive representation of local image regions (Guo et al. 2010). Discriminative completed LBP (DCLBP) is a further improvement of conventional LBP to capture both the sign and magnitude of local differences and select the most informative features (Guo et al. 2012). Ahmed et al. (2011) proposed combining LBP with template matching and support vector machines, and later, Ahmed et al. (2014) integrated LBP with local ternary patterns and local directional patterns to improve discriminative power. Despite these advances, LBP-based methods have not yet been widely validated for weed seed discrimination in quarantine contexts and remain primarily a research-stage tool (Lei et al. 2017).

Collectively, morphology-based identification methods have significant drawbacks for quarantine use. They are time-consuming, require skilled technicians to obtain reliable results, and struggle with heavily processed or damaged seed material commonly found in commercial consignments. Even though morphological identification techniques have limitations, they are essential for first-line detection of regulated species at quarantine ports, especially for large shipments where rapid assessment is needed before any molecular confirmation step occurs. Consequently, a need to identify and implement rapid and complementary molecular tools in weed seed detection is highlighted.

Molecular Approaches

Several molecular techniques for plant identification have been developed and applied over the past several decades. These methods provide reliable species identification when phenotypic traits are insufficient. In weed science, they have been used for weed seed identification and have potential for application in quarantine weed detection at ports of entry. Many are described below.

DNA Barcoding

DNA barcoding, based on an analysis of a specific gene region, provides reliable identification (Shen et al. 2013; Whitehurst et al. 2020).

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Table 1. Comparative analysis of species detection methods for weed seed identification in plant quarantine settings.[a]

Method	Principle	Time required	Sensitivity[b]	Specificity[c]	Equipment needed	Expertise required	Cost level US$[d]	Suitability for Quarantine	Key advantages	Key limitations
Morphological identification	Visual traits (e.g., seed size, shape, color)	Minutes to hours	Low to moderate	Low	Basic tools (e.g., microscope)	Expert taxonomist/seed analyst	Very low (<1/sample)	Moderate	Simple, inexpensive	Difficult for morphologically similar, damaged or immature samples
Grow-out test	Seed germination and seedling traits	21–28 d	Moderate	Moderate	Growth chamber, greenhouse	Plant identification expertise (moderate)	Low (2–5/sample)	Low	Confirms viability	Very slow, dormancy issues
Spectroscopy/HSI	Spectral and image analysis	Minutes	High	Moderate to high	NIR spectrometer/ hyperspectral imaging system	Specialist in spectroscopy and data analysis	Very high (0.5–2/sample running cost, but $50,000–$200,000 per instrument)	Moderate	Rapid, nondestructive	High cost, needs a reference database
DNA barcoding (PCR + sequencing)	Amplify and sequence targeted genes or gene regions	1–3 d	High	Very high	Thermocycler and sequencing platform	Molecular biologist and bioinformatics skills	High (10–20/sample including sequencing)	Moderate	Accurate species identification	Time-consuming, costly
qPCR	Fluorescent detection during DNA/RNA amplification	2–4 h	Very high	Very high	qPCR machine	Trained molecular technician	High (5–15/reaction)	Moderate	Fast, quantitative	Requires lab infrastructure
Melt curve analysis	Tm profiling of selected amplicon	2–3 h	High	High	qPCR machine	Moderate molecular skills (data interpretation needed)	Moderate (3–8/reaction)	High	No sequencing needed	Requires reference Tm profiles
Bar-HRM	DNA barcoding coupled with high-resolution melting	2–3 h	Very high	Very high	HRM enabled qPCR	Moderate molecular skills and basic data analysis	Moderate (3–5/reaction)	Very high	Rapid, highly accurate	Needs initial validation/ database
LAMP	Isothermal amplification (60–65 C)	<30 min (amplification only) + 30–50 min if gel based detection	Very high	High	Simple heater or block	Basic molecular training (field adaptable)	Low to moderate (2–5/ reaction)	Very high	On-site application, rapid	Primer design complexity
RPA	Isothermal amplification (37–42 C)	15–30 min (amplification) + 5–10 min LFS readout	Very high	High[e]	Minimal/portable heat block	Minimal training (field technicians)	High (8–15/reaction; reagent kits are expensive)	Very high	Very fast, onsite detection	Expensive reagents

[a]Abbreviations: Bar-HRM, barcode DNA high resolution melting; HSI, hyperspectral imaging; LAMP, loop-mediated isothermal amplification; LFS, lateral flow strips; PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; RPA, Tm, melting temperature.
[b]Sensitivity refers to the ability to detect true positives (low false negatives).
[c]Specificity refers to ability to exclude non-targets (low false positives).
[d]Costs are approximate and vary by region, supplier, and batch size.
[e]Low specificity if nonspecific amplification risk if primer design is suboptimal.

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In this process, DNA is extracted from the sample, and the target DNA region (amplicon) is amplified using polymerase chain reaction (PCR). After confirming a successful PCR using gel electrophoresis, the resulting amplicon is sequenced. The sequence is then queried against reference databases such as GenBank or the Barcode of Life Data System (BOLD) to identify the species (Bruni et al. 2010). This process is relatively time-consuming because species confirmation often requires PCR amplification and sequencing of multiple gene regions (Foster et al. 2013). Although DNA amplification and sequencing can be performed simultaneously using next-generation sequencing platforms, their application depends on the availability of adequate infrastructure and technical expertise. In many countries, limitations in cost, facilities, technical capacity, and awareness restrict the use of such methods in quarantine screening centers.

Individual or combined genomic loci are employed as candidate barcode sequences for plant identification, but the most appropriate DNA barcode for particular taxonomic groups is critically important in species identification. Wang et al. (2017) evaluated six candidate loci, *atpF* intron, *matK*, *ndhK-ndhC*, *psbE-petL*, ETS, and ITS, to identify invasive grasses in eastern Australia that are co-occurring with native grasses often mistaken for the invasive types. These loci were screened because the standard universal barcodes provide insufficient resolution for interspecific species identification in Poaceae. The study results suggested that *matK* and *atpF* are dual loci that can be used to distinguish several invasive grass species from native species. On the other hand, PCR success of the ETS locus was low but was effectively used to distinguish two important invasive grass species. For *Solanum* L. weed species, Zhang et al. (2013) showed that the chloroplast regions *ndhF* and *trnS-trnG*, combined with the nuclear gene *waxy*, provide the resolution needed to distinguish invasive *Solanum* from native congeners. Distinction of invasive *Solanum* species from native species is important at quarantine points where morphological separation is unreliable.

Although PCR is widely regarded as a standard identification method for most DNA-based approaches, it requires skilled personnel, complex read-out procedures, and costly equipment such as electrophoresis systems and visualization equipment. These requirements limit its accessibility for non-scientist users in government agencies and border inspection posts (Liu et al. 2018).

Isothermal Amplification Methods

Isothermal amplification includes a group of nucleic acid amplification techniques that operate at a constant temperature, thereby eliminating the thermal cycling requirements of conventional PCR as well as personnel requirements (training and labor). Developed as an alternative to thermocycler-dependent methods, isothermal amplification methods (IAMs) use distinct enzymatic mechanisms, including recombinases, helicases, and strand-displacing polymerases, to achieve rapid and sensitive DNA or RNA replication (Nieuwkerk et al. 2020). Among the different techniques, loop-mediated isothermal amplification (LAMP) and recombinase polymerase amplification (RPA) are methods commonly used for plant detection (Panozzo et al. 2023; Lei et al. 2017). The inherent simplicity of IAMs, coupled with their compatibility with portable detection platforms such as lateral flow assay strips, has driven their increasing adoption in resource-limited settings (Nieuwkerk et al. 2020). Collectively, the IAM, LAMP, and RPA techniques have demonstrated diagnostic sensitivity and specificity comparable to that of PCR, while offering substantial advantages in terms of cost, speed, and equipment requirements.

Loop-Mediated Isothermal Amplification

LAMP is emerging as a promising tool in clinical, agricultural, and environmental diagnostic contexts. DNA amplification via LAMP can be optimally operated in the 60 to 65 C temperature range. The method does not use a heat denaturation step as in PCR; instead, it uses a strand-displacing polymerase (such as *Bst* polymerase; a specialized enzyme that synthesizes DNA while displacing the downstream non-template strand), which allows isothermal amplification without the need for thermal cycling (Notomi et al. 2015). LAMP requires four to six primers targeting six to eight distinct loci of the template. Primer designing in LAMP is much more complex than in standard PCR, which uses only two primers. This complexity confers high specificity and enables rapid amplification without thermal cycling (Nieuwkerk et al. 2020; Notomi et al. 2015). This design complexity is a key consideration when developing LAMP assays for weed species that need to be quarantined because validated primer sets must be established and tested before field deployment. LAMP assays can be detected in a few ways, such as turbidity-based LAMP detection. The change of turbidity in the reaction mixture due to the precipitation of magnesium pyrophosphate as a byproduct of nucleic acid replication is measured using a turbidity meter (Novi et al. 2025). In fluorescence-based LAMP detection methods, fluorescent dyes, probes, or nanoparticles act as signal transducers to detect amplicons in the sample (Becherer et al. 2020). Colorimetric methods based on pH-sensitive dyes are also used in LAMP detection assays, in which the pH change from H^+ ions produced by DNA polymerization lowers the buffer pH, leading to a color change in the pH indicator being used (Tanner et al. 2015). Intercalating dyes (e.g., SYBR Green I), pH-sensitive dyes, metal-indicating dyes, etc., are commonly used in the process (Novi et al. 2025).

Research on LAMP in weed detection has thus far been limited. However, a few studies are worth mentioning. Panozzo et al. (2023) implemented the technique to rapidly detect target-site herbicide resistance in ryegrass (*Lolium* spp.) revealing that LAMP is useful for detecting target-site resistance with target genes with less genetic variability. LAMP targeting the chloroplast *trnL–trnF* intergenic spacer region can rapidly identify several poisonous blue rocket (*Aconitum* L.) species, providing a potential screening method for the diagnosis of Aconitum poisoning in emergency medical care, particularly in cases where plant material has been accidentally ingested by humans. Although the template DNA was processed and degraded, LAMP performed effectively, and the overall detection procedure was rapid, requiring only 30 min (Kitamura et al. 2019). Agarwal et al. (2022) implemented the LAMP assay as a diagnostic method for rapidly identifying an invasive plant pest, fall armyworm (*Spodoptera frugiperda* J. E. Smith), using the mitochondrial gene *COI* to accurately identify the insect. The method was found to be highly specific, rapid (less than 20 min to perform), and reliable in identifying fall armyworm. Even though the method has been applied to insect pest identification, similar procedures can be implemented for specific weed detection (Figure 1).

Recombinase Polymerase Amplification

Recombinase polymerase amplification (RPA) is uniquely operated at temperatures as low as 37 C, rendering it particularly amenable to field-based and point-of-care applications (Nieuwkerk et al. 2020). It is an isothermal amplification technique that provides rapid, sensitive, and specific molecular identification without the need for thermocycling equipment. It has been

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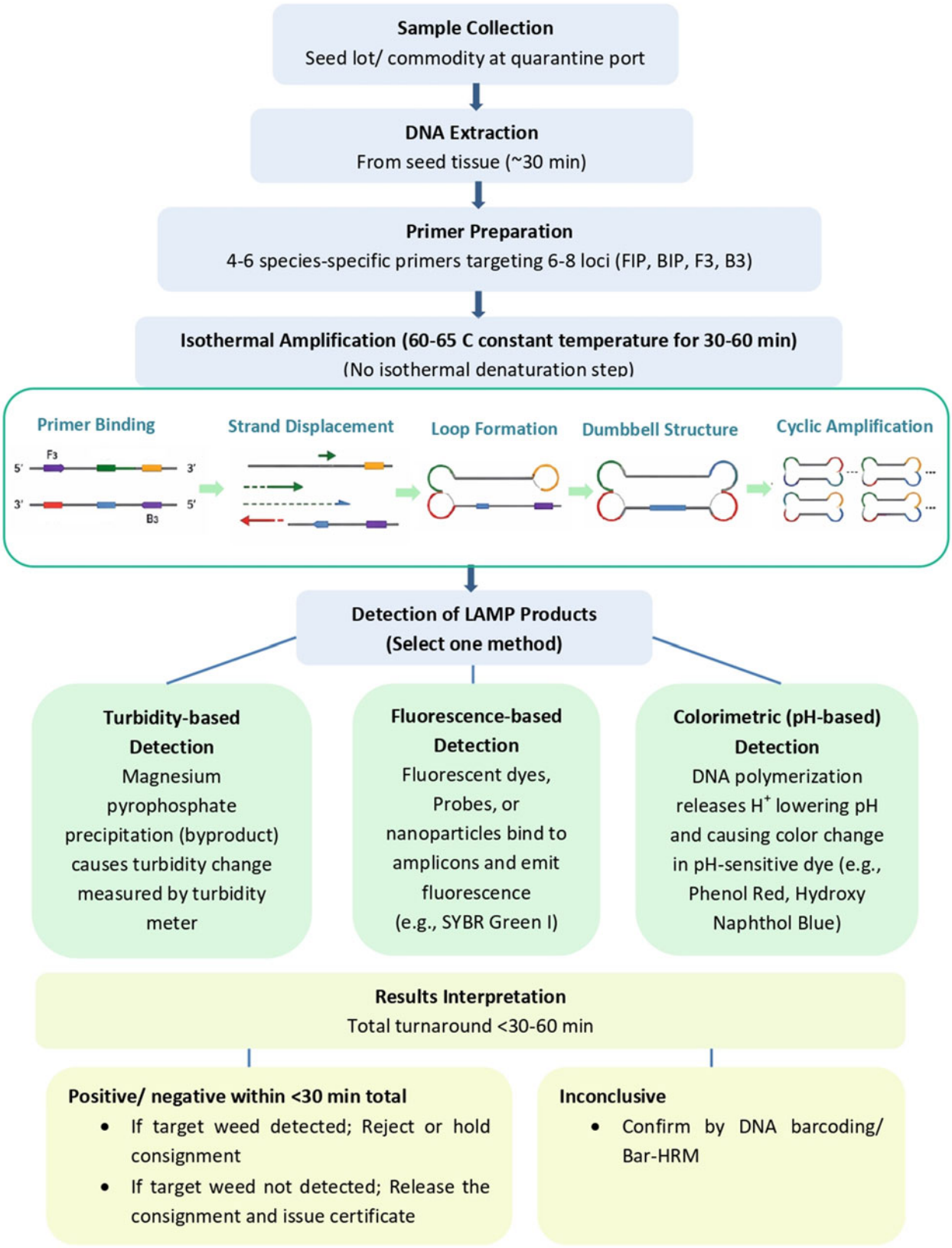


Figure 1. Conceptual framework illustrating the loop-mediated isothermal amplification (LAMP) assay workflow as applied to weed seed detection within a plant quarantine screening context.

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noted for its accuracy and specificity of molecular diagnoses (Loo et al. 2013). Unlike conventional PCR, RPA performs a reaction at a steady temperature below 40 C using recombinase enzymes to separate double-stranded template DNA and facilitate primer binding. As a result, a nucleoprotein complex is formed by a strand-displacing DNA polymerase, such as *Bsu* or *Bst* DNA polymerases, along with recombinase and single-stranded binding proteins, enabling primer extension and displacement of the non-template strand, leading to target amplification within 3 to 20 min (Lutz et al. 2010). Several studies (e.g., Lobato and O'Sullivan 2018; Piepenburg et al. 2006) have obtained results in even less than 30 min. This makes RPA significantly faster, more economical, simpler, and more effective than conventional PCR amplification. Less complex equipment, such as lateral flow strips, can be used to observe amplification outcomes by technicians without molecular biology training.

Although RPA was designed for use in the fields of in vitro diagnostics, virus detection, and pathogen verification, it is also emerging as a promising tool in the surveillance of quarantine monitoring services. The RPA–lateral flow strip assay has been developed as a rapid on-site plant species identification tool. For example, Liu et al. (2018) used the method to successfully detect the presence of Japanese pagoda tree [*Styphnolobium japonicum* (L.) Schott] adulteration in Ginkgo (*Ginkgo biloba* L.) herbal products, demonstrating the capacity of RPA for species-specific plant material authentication. The rapid identification of quarantine nightshade (*Solanum* L.) weeds is difficult due to the morphological similarities of seeds and seedlings. Lei et al. (2017) used RPA technology to rapidly identify silverleaf nightshade (*Solanum elaeagnifolium* Cav.), an aggressive poisonous quarantine weed. The approach has been recognized as a fast and easy-to-implement method that features recommendations for the RPA-based assay for on-site identification of weed species that are morphologically similar to a species of interest (Figure 2).

Real-time Quantitative PCR

Quantitative (qPCR) is a molecular DNA or RNA quantification technique that uses fluorescent reporter molecules during the amplification process. In qPCR, fluorescent signals are detected and measured at the end of each amplification cycle. Thus, the turnaround time for delivering results is within 2 to 4 h. Conventional PCR further involves postamplification electrophoresis, which is not necessary in qPCR, and requires additional time and a risk of contamination.

In qPCR, each sample undergoes amplification with a fluorochrome that fluoresces only upon production of the specific target product, enabling real-time quantification without post-amplification gel electrophoresis (Gachon et al. 2004). Among available chemistries, SYBR Green is the most cost-effective option suitable for initial assay development, whereas *Taq*Man probes offer greater specificity for discriminating closely related weed species (Chao 2008). The ability of qPCR to detect trace amounts of weed seed contamination make it well-suited for detecting high-priority regulated species where zero-tolerance thresholds apply. Furthermore, the process is quick and needs a turnaround time of just 2 to 4 h. However, qPCR requires thermocycling equipment and trained personnel, which limits its deployment to regional or national laboratories rather than field-based inspection posts.

Quantitative PCR has been used in plant-based detection studies and has a good potential for effective use in weed identification at quarantine centers. qPCR has been used to detect cannabis (*Cannabis sativa* L.) in unknown materials in forensic laboratories, and the method has been proven to provide a quick and accurate way to confirm the presence of cannabis in unknown materials (Johnson et al. 2013). The application is highly relevant to the quarantine context because cannabis is a regulated plant in many countries, and its seeds are indistinguishable from other related hemp species. Ferreira et al. (2016) also used qPCR to develop a method for detecting cereal adulterants in commercial coffee products. That study highlights the versatility of the technique for species-specific detection in complex matrices, such as detecting seeds in bulk commodity shipments. The qPCR assay enables rapid species confirmation (within hours) and thus can replace lengthy grow-out tests. The method can be adapted for quarantine species by targeting species-specific primers and probe kits validated against local flora. Consequently, the rapidity, sensitivity, specificity, and high range of quantification are major advantages over conventional PCR, making it a superior technique to deploy as a detection tool in plant quarantine laboratories.

DNA Melt Curve Analysis

Melt curve analysis (MCA) is a post-PCR analysis technique that evaluates the biophysical properties of amplified DNA (Reed and Wittwer 2004, Ririe et al. 1997; Wittwer et al. 2024). After PCR amplification, double-stranded DNA (dsDNA) intercalated with a fluorescent dye is gradually heated to a defined temperature range. An optical system continually monitors this fluorescence. As the temperature rises, the dsDNA gradually denatures and the dye is released, causing a drop in fluorescence. While the dye is bound, fluorescence is strong; as the dye goes into the solution, the fluorescence weakens. This is a gradual process, and when the dsDNA completely separates into two single strands, an immediate drop in fluorescence occurs. This immediate drop is sharpest near the melting temperature (Tm) of the PCR product. Each DNA has a characteristic Tm. The temperature at which 50% of the DNA is single-stranded forms a melting curve profile for each amplicon, the resulting of which serves as a unique molecular fingerprint for species identification.

Vulchi et al. (2021) used MCA for species identification by developing assays to identify lepidopteran pest species in almonds and pistachios samples. Species-specific PCR primers were used to amplify target gene regions from unknown samples and the resulting melt curves were compared to reference profiles from authenticated specimens. The researchers were able to identify each species by comparing their Tm values to the reference library, enabling discrimination among morphologically similar pest species without gene sequencing. This technique could be similarly developed for quarantine weed detection at quarantine centers by designing species-specific primers for target weed species and establishing reference melt curve libraries from authenticated weed collection, thereby enabling confirmatory identification within a few hours.

Barcode DNA High Resolution Melting Analysis

Barcode DNA high resolution melting (Bar-HRM) is a post-PCR molecular technique that combines DNA barcoding with high-resolution melting analysis to detect genetic variation in DNA sequences with high sensitivity (Pereyra et al. 2012). As described for MCA, Bar-HRM evaluates species-specific melt curve profiles after PCR amplification. In contrast to MCA, Bar-HRM uses brighter fluorescent dyes at higher concentrations, instruments with finer temperature resolution (increments as small as 0.01 C), and more sophisticated curve-normalization software (Grazina et al. 2021). Hence, Bar-HRM is sensitive enough to distinguish

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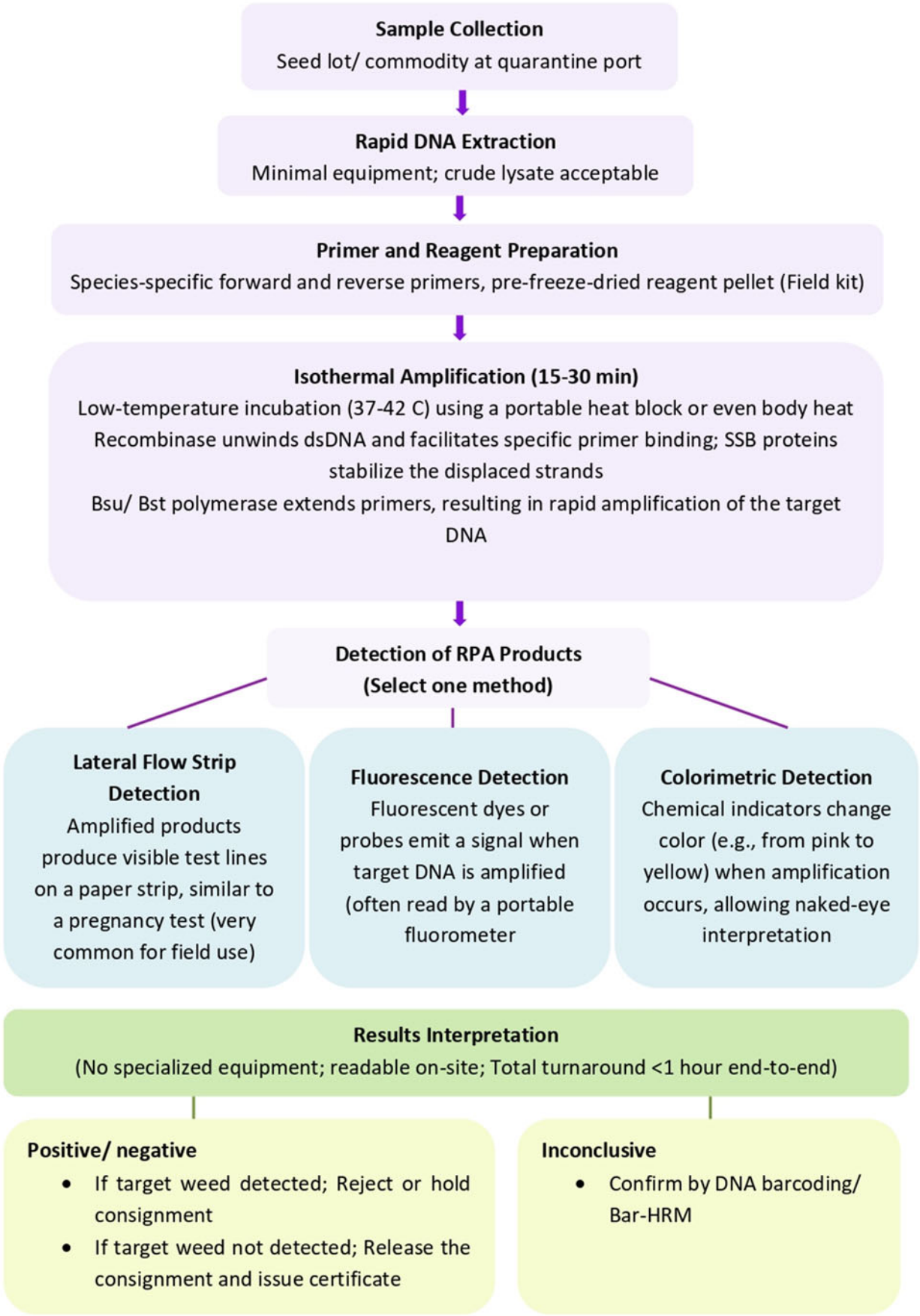


Figure 2. Conceptual framework illustrating the recombinase polymerase amplification (RPA) assay workflow as applied to weed seed detection within a plant quarantine screening context.

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sequences that differ by as little as a single nucleotide, without the need for DNA sequencing. The method operates entirely in a closed-tube system, eliminating post-PCR handling and the associated contamination risk. For quarantine applications, this combination of high sensitivity, moderate cost, and closed-tube operation makes Bar-HRM a strong candidate for national reference laboratories where definitive species confirmation is required. Compared to MCA, Bar-HRM demands more sophisticated instrumentation and software, but offers substantially greater discriminatory power for closely related weed species whose melt profiles may overlap under standard MCA conditions.

Several studies have uses the Bar-HRM process for species authentication. For example, Kumari et al. (2025) implemented Bar-HRM to rapidly and precisely identify 12 selected weed species in Sri Lanka, and Jaakola et al. (2010) used it for berry (Ericaceae, Crossulariaceae, and Rosaceae) species authentication. Madesis et al. (2012) extended the method to detect lupine (Fabaceae) in soybean products using the universal *trnL* chloroplast marker combined with a direct PCR kit, which requires no prior DNA extraction, substantially simplified the workflows. Osathanunkul et al. (2015) further validated Bar-HRM for authenticating three medicinal Acanthaceae species [holly mangrove (*Acanthus ebracteatus* Vahl.), Kariyat (*Andrographis paniculata* (Burm.f.) Wall. ex Nees), and Snake Jasmine (*Rhinacanthus nasutus* (L.) Kurz)] that are commonly available in drug stores in Thailand. These studies demonstrate the applicability of Bar-HRM across diverse plant families. Compared to other techniques for analyzing genetic differences, HRM analysis operates in a closed-tube system, requires no manual post-PCR handling, and has a low per-sample processing cost (Hofinger et al. 2009).

Bar-HRM has great potential as a process for precise discrimination of weed species. The method can be implemented to accurately and rapidly detect quarantine weeds in a three-step process. First, select the appropriate barcode loci (e.g., *trnL*, ITS, or species-specific markers) that provide sufficient interspecific variation to discriminate target weed species from morphologically similar weeds, crops or native plants. Second, establish a reference HRM profile library from authenticated specimens of target species. Third, validate the assay using simulated quarantine samples, such as weed seeds spiked into commodity matrices, to confirm sensitivity and specificity under realistic inspection conditions. This approach requires moderate molecular biology expertise and standard qPCR equipment, making it accessible to national quarantine laboratories.

Comparative Analysis of Various Detection Methods for Quarantine Facilities

Which detection methods a quarantine facility chooses for invasive weed detection depends on several factors, including availability of infrastructure, time (turnaround time), cost, and the sensitivity of identification or detection of the target species. Comparative analysis of the different detection methods is important when selecting a suitable method or methods for quarantine purposes (Table 1).

The cost per reaction and level of expertise required for the various detection methods vary considerably. Among them, morphological examinations and sieving methods are important for initial screening at high-volume ports due to their low cost and speedy detection. Yet these methods alone are incapable of species-level identification of morphologically ambiguous samples. IAMs such as RPA and LAMP are the most feasible molecular techniques for deployment at resource-limited ports because of their minimal equipment requirements and compatibility with lateral flow readouts. For national and regional quarantine laboratories, qPCR and Bar-HRM methods are recommended because they can confirm species with high accuracy within a working day. Molecular techniques such as Bar-HRM offer a balanced combination of accuracy, speed, and moderate cost, while isothermal techniques such as LAMP and RPA provide rapid and field-deployable alternatives that are suitable for quarantine inspections. Nevertheless, DNA barcoding remains the best method for reference identification and database development, although its turnaround time is 1 to 3 d, which limits its utility for routine clearance decisions.

The selection of detection methods is highly dependent on several factors such as national regulatory frameworks, tolerance limits, and available infrastructure. For weed species that have zero-tolerance policies (silverleaf nightshade in Australia, purple witchweed in many African countries, and dodder in the European Union), methods must be capable of detecting a single seed within large consignments, and therefore morphological screening alone may not be effective. In such cases, molecular techniques such as qPCR and LAMP are reliable and suitable, whereas for species with tolerance thresholds exceeding 0.1% by weight, conventional morphological screening combined with confirmatory germination assays may still be adequate under current regulatory conditions.

Disparity in infrastructure among quarantine systems in high-income and low-income countries is another important factor when adopting weed seed detection systems. Many border inspection points in lower-income countries lack stable electricity, appropriate cold storage for reagents, and access to trained molecular specialists. Hence, thermocycler-based methods are impractical. In such locations, isothermal amplification methods are more feasible. Isothermal techniques function at constant, relatively low temperatures (a water bath is sufficient) and results can be obtained via lateral flow strips or simple screening methods that require no specialized equipment or personnel to interpret. However, unavailability of standards for molecular detection methods for weed seed identification is a major regulatory gap that restricts the acceptance of molecular diagnostic evidence in international phytosanitary trade disputes. Unlike the guidance provided for plant pathogens in the International Standards for Phytosanitary Measures, internationally harmonized molecular detection standards are absent for weed seeds. Hence it is a significant regulatory gap that currently limits the legal admissibility of molecular evidence in international phytosanitary trade disputes.

Synthesis and Conclusion

This review has provided an overview of the literature on various techniques that can be adapted for the detection and accurate identification of plant seeds in a plant quarantine context. Morphological methods for identification are accessible and rapid, but they have limited usability for processed seed material and are generally unsuitable for definitively identifying species when morphological characteristics among them are difficult to discern. Additionally, because weeds are likely contaminated as seeds within commercial products or living plant materials, separating them for seed morphology studies is challenging. As a result, inspections have often resorted to grow-out tests to observe seedling characters, which require varying time periods depending

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on weed species. However, accurate identification requires more time until vegetative and reproductive traits are visible. Molecular approaches on the other hand are more specific and sensitive in detection and identification, though they vary in cost, required expertise and deployment feasibility.

Among the molecular methods described here, Bar-HRM analysis shows considerable promise for supporting quarantine decision-making at the national laboratory level, owing to its high sensitivity to detect single nucleotide differences, comparatively moderate cost, lack of post-PCR manipulation, and quick turnaround time. However, use of Bar-HRM in quarantine weed or seed detection is limited. Most published validations have been conducted only in food authenticity or pharmaceutical contexts. Therefore, broader validation of Bar-HRM on quarantine-relevant weed species and commodity matrices is required before adaptation is recommended. RPA and LAMP methods are similarly highly applicable as rapid, field-base methods, yet weed-specific assay development is needed before they are used in making quarantine decisions.

As summarized earlier and in Table 1, method selection should be guided by a tiered framework that matches each approach to the facility type and operational context. It is essential to consider balancing detection speed with the level of confidence in species identification when selecting a detection method. Morphological screening provides rapid results within minutes and at minimal cost; however, it may be insufficient for confirming species identity when closely related or morphologically ambiguous taxa are involved. Consequently, DNA barcoding offers highly reliable identification but requires extended processing times (typically 1 to 3 d) and access to specialized laboratory infrastructure. RPA and LAMP are able to partially replace PCR-based methods and deliver results within 30 to 60 min and at a moderate cost. However, their application in border inspection is currently limited by the availability of validated species-specific assays for most quarantine weed species. Molecular techniques such as qPCR and Bar-HRM provide a balance between accuracy and turnaround time, yielding results within 2 to 4 h in well-equipped laboratory settings. Hyperspectral imaging has high potential but is predominantly used in research at present, requiring further infrastructure investment and species-specific validation before its routine adoption can be recommended. Collectively, these trade-offs highlight the necessity of adopting a tiered and context-dependent strategy for method selection, as described in the framework below.

Apart from molecular methods, hyperspectral imaging can be effectively used for rapid, nondestructive, high-throughput screening of large seed consignments, but little research on it has been conducted thus far to develop it as a tool in weed identification and quarantine. As a result, comprehensive and validated spectral reference libraries are unavailable to analyze results. Collectively, these trade-offs highlight the necessity of adopting a tiered and context-dependent strategy for method selection, rather than relying on a single, universal approach to detection.

This review has direct relevance to quarantine policymakers and inspectors at governmental plant quarantine agencies worldwide. Compiling and critically evaluating available detection methods in a single framework are important for providing evidence in making informed decisions about method adoption and infrastructure investments. The comparative table (Table 1) can serve as a practical guide in selecting methods appropriate to their specific operational contexts from onsite, rapid tests at land borders to high-throughput laboratory platforms at major seaports and airports. For example, the National Plant Quarantine Service in Sri Lanka is actively seeking to modernize its weed detection practices. Our findings suggest that a combined approach in which morphological prescreening occurs with targeted molecular confirmation for suspect samples may offer the most practical and cost-effective path forward.

Based on the comparative analysis presented in this review, a practical three-tier framework is proposed for weed seed detection in plant quarantine systems. Tier 1 is from the port of entry to all facilities, initial screening by morphological inspection, sieving, and gravity separation. These methods are rapid, inexpensive, and do not require laboratory infrastructure and are applicable for identifying many regulated weeds. This method is also effective for clearing clearly compliant consignments and identifying suspect samples that require further analysis. Tier 2 is applicable for regional quarantine laboratories. If any suspect sample is identified at Tier 1 it can be subjected to same-day molecular confirmation using LAMP or RPA. These IAMs require minimal equipment, are relatively cost-effective, and can be performed by trained personnel without advanced molecular expertise. When validated assays are available, they represent the most practical molecular tools for routine quarantine diagnostics. Tier 3 is the last step and is implemented at national reference laboratories. Advanced methods such as DNA barcoding, qPCR, and Bar-HRM provide definitive species-level identification, support regulatory decision-making, and contribute to reference database development. These methods are feasible at national reference laboratories because they require specialized facilities and expertise, and their turnaround time of 1 to 4 h is better suited to critical decision-making rather than routine screening. This tiered approach recognizes that no single detection method is universally optimal, thus effective quarantine systems depend on integrating complementary techniques tailored to available resources and the specific risk context.

Through this review, we have identified several important research gaps and future directions that should be addressed promptly. First, there is a critical need for the development and public deposition of comprehensive reference DNA barcode libraries for agriculturally important weed species, particularly for tropical and subtropical floras that remain underrepresented in global databases. Second, the performance of molecular methods must be validated specifically on degraded or processed seed DNA. Third, the development of multiplexing approaches that can simultaneously screen for multiple quarantine weed species in a single reaction. Multiplexing of several samples is more practical for ports due to their handling of diverse commodities. Fourth, the integration of machine learning with hyperspectral imaging is another promising tool for rapid, nondestructive species identification. It warrants further investigation in quarantine-specific settings. Addressing these gaps will be essential for realizing the full potential of advanced detection technologies in global plant biosecurity.

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