Fluorescent Tracers by Soybean

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Systemic seed treatment uptake was investigated in seeds and seedlings using fluorescent tracers to mimic systemic agrochemicals. Soybean was used as the model as soybean has the permeable seed coat characteristic to both charged and noncharged molecules. The purpose of the paper is to (1) screen 32 fluorescent tracers and then use optimal tracers for seed and seedling uptake, (2) investigate varietal differences in seed uptake, (3) examine the distribution of tracer uptake into 14-day-old seedlings, and (4) study the relationship between seed treatment lipophilicity, measured as log P on seed and root uptake. The major chemical families that displayed both seed and seedling uptake were coumarins and xanthenes. Seed uptake of coumarin 120 ranged from 1.1% to 4.8% of the applied seed treatment tracer from 15 yellow-seeded varieties. Rhodamine B, a xanthene compound uptake in seedlings, showed translocation from the applied seed treatment to all seedling tissues. Most of the tracer was measured in the hypocotyl and root, with lesser amounts in the epicotyl and true leaves. Log P is well documented in the literature to model systemic uptake by roots, but log P of the tracers were not related to seed uptake.

Keywords: fluorescent tracer ; systemic uptake ; soybean ; in vivo imaging system (IVIS)

1. Introduction

Crop seeds are treated commercially by the seed industry or on the farm to protect seeds and seedlings from attack by insect pests and pathogens that cause plant diseases [1]. Active seed treatment ingredients may have contact activity or be systemic in nature. Compounds with contact activity are restricted to control of pests in the immediate vicinity of the sown treated seed. In contrast, a compound with systemic activity protects in the immediate vicinity and is also translocated within the plant [2]. Thus, systemic movement allows the protection of plants during the early stages of seedling growth after emergence. The agronomic benefit of using systemic seed treatments is to reduce the need for foliar applications for early season pest management, thus avoiding a field operation that may not be possible or be delayed due to weather-related events and/or wet soils. Therefore, seed treatments are used globally for early season pest management and have less potential environmental impact than foliar applications due to lower pesticide usage per hectare [3].

The physiochemical properties required for a systemic seed treatment to permeate through the seed coat to the embryo and ultimately be taken up into the seedling and transpiring leaves are still not well understood. One pathway for systemic uptake in seeds is for a compound to diffuse through the seed coat to the embryo during imbibition. For this to occur, the seed coat must be permeable to the specific compound. Seed coat permeability has been previously investigated on several crops using fluorescent tracers [4][5][6]. Taylor et al. [5] reported that the passage of organic compounds applied as seed treatments to the embryo during imbibition is dependent on the chemical properties of the treatment and crop species. As a result of this research, crop species were grouped into three categories based on seed coat permeability, those with permeable, selectively permeable and nonpermeable seed coats [4][5]. Crops with a permeable seed-coats, including soybean (Glycine max (L.) Merr.), snap bean (Phaseolus vulgaris L.), and pea (Pisum sativum L.), were reported to be permeable to both nonionic and ionic compounds, while selectively permeable crop seeds, including corn (Zea mays L.), onion (Allium cepa L.), tomato (Solanum lycopersicum L.), and pepper (Capsicum annuum L.) were only permeable to nonionic compounds. Cucumber (Cucumis sativus L.) and lettuce (Lactuca sativa L.) seeds were categorized as having nonpermeable seed coats as they were not permeable to either nonionic or ionic charged compounds. In these experiments, systemic tracer translocation was observed under long-UV light, eliminating the use of pesticides and radioactively labeled compounds. The nine fluorescent tracers used in these experiments varied by ionic charge (nonionic, cationic, or anionic) [4][6]. Investigation of seed uptake and systemic activity using fluorescent tracers with a range of physio/chemical properties will help to expand our knowledge and utility of these tracers in seed technology research. Moreover, an expanded set of tracers could aid in understanding the relationship between seed uptake and root uptake.

Both nonionic (coumarin 151) and ionic (rhodamine B) tracers diffused from treated soybean seed, with a permeable seed coat, to the embryo after sowing in a moist medium [6]. In a separate experiment with a nonionic fluorescent tracer, coumarin 120, the maximum or saturated seed uptake of two yellow-seeded varieties was more than 50% greater than a black-seeded variety, illustrating varietal differences between genotypes [5]. Due to these varietal differences, additional research is needed to understand agrochemical uptake in seeds more fully.

The fluorescent tracer coumarin 151, used to investigate uptake into seedlings in snap bean and cucumber, was found in the xylem vessels of all seedling structures, including the roots, hypocotyl, cotyledons, petiole, and true leaves [7]. These observations indicate that systemic uptake of coumarin 151 is by apoplastic or acropetal movement. A more recent report showed uptake of rhodamine B uptake in snap bean seedlings [8]. Collectively, fluorescent tracers have great utility to assess seed coat permeability and systemic uptake into plants.

2. Datas

As previous research has demonstrated, fluorescent tracers are convenient tools to visualize the movement of compounds in animals, plants, and seeds for qualitative and quantitative measurements. Fluorescence imaging systems are also useful tools that can quantify excitation and emission signals over a wide range of wavelengths. The in vivo imaging system (IVIS) was developed for in vivo fluorescence and bioluminescence imaging. IVIS consists of a stationary charge-coupled device (CCD) imaging camera with illumination and a set of excitation filters from 415–760 nm in 30 nm bandwidths and a set of emission filters from 490–850 nm in 20 nm bandwidths [9]. IVIS is used in small animal imaging for nondestructive, noninvasive, internal imaging of fluorophores [10]. In addition, the IVIS spectrum system is also used in plant science research [11][12][13], and has potential for fluorescence imaging of seeds of large-seeded crops.

Several physical/chemical properties of an organic compound contribute to systemic activity and plant root uptake. Much of our understanding and rules that govern the ability for uptake was first illustrated for oral pharmaceuticals and described as Lipinski's rule of five, or simply, the rule of five (RO5) [14]. A compound having chemical properties satisfying the RO5 has potential pharmacological or biological activity as an orally active drug in humans [14]. The RO5 approach used in pharmacology was quickly adopted with some modifications to profile agrochemical uptake, and several new "rules" were established by Briggs, Carr, Tice, and Hao, cited by Jampilek (2016) [15]. Of particular interest to seed science were the properties described by Clarke, known to influence the absorption and distribution of agrochemicals in crop plants, termed the rule of two. The parameters or criteria important for the rule of two are molecular mass from 200–400, log $P \leq 4$, and hydrogen-bond donors ≤ 2 [15][16].

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