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A BIBLIOMETRIC REVIEW OF RESEARCH TRENDS IN SALICYLIC ACID USES IN AGRICULTURAL AND BIOLOGICAL SCIENCES: WHERE HAVE BEEN STUDIES DIRECTED?

Muhittin KULAK[1, 2]

[1]Department of Biology, Faculty of Arts and Sciences, Aralık University, Kilis 7, Turkey
[2]Department of Nursing, Yusuf Serefoglu School of Health, Aralık University, Kilis 7, Turkey

Corresponding author email: muhyttynx@gmail.com

Abstract

Plants encounter numerous biotic and abiotic stress factors during their life cycle, either simultaneously or at different times. For the exogenous chemicals applied to improve stress induced perturbations, salicylic acid was found to be mostly preferred and used as a growth regulator. The objective study was to examine the profile of original and review articles under the topic of salicylic acid using bibliometric analysis. The VOSviewer tool was used to visualize the results. The documents including salicylic acid keyword but limited to agricultural and biological sciences were extracted from Scopus database. In this context, 1.422 documents relevant and available peer-reviewed publications were analysed. According to the systematic analysis, 4688 authors, 2880 keywords and 84 countries were determined. The annual trends of publications in the field of research, considering the number of documents, number of authors, levels of collaborations among authors and countries, year publications and core publishing journals were analysed. Finally, the explanations regarding with the improved response via salicylic acid in plant systems were briefly represented in the present study.

Key words*: salicylic acid, agricultural and biological sciences, bibliometric analysis.*

INTRODUCTION

People have always been tied to plants in order to be the source of the food. With the development of modern and industrial life, there has been a significant increase in both the quantity and quantity of plant products used by humans. The rapid increase in world population (150 babies/ min) has further increased the need for plant products. It has been estimated that people have been struggling with plant growth, yield and quality for a long time for various purposes such as nutrition, clothing, medicine and energy. To control and improve plant growth, yield and quality, plant scientists offer significant contributions to solve the problems of the rapidly increasing world population. Whether future generations can raise their standard of living or continue at all as it is, it will be possible only through the further development of plant physiology and the application of modern methods to agriculture, forestry and industry. The main objectives are the development of methods by which plant scientists can easily and broadly apply the main principles they have gained from various experiments in economics and agriculture. For example; increase in fruits and flower yield, modernization of fertilization and irrigation techniques, storage, transportation and marketing of agricultural products are very important for the healthy and prosperous sustainability of our daily life (Bozcuk, 2004). Numerous and diverse laboratory, pot and field experiments have been set up to solve these and similar problems. In addition to agricultural applications, different harvesting times and post-harvest practices have been carried out to obtain plants at the desired level and quality. In addition, plants are exposed to biotic and abiotic stress factors and the changes that occur in plants are examined. A variety of external practices are being implemented to eliminate the negative consequences of stress. In this study, salicylic acid (SA), which is often used as an exogenous growth regulator, was discussed. The structures of salicylic acid, the mechanism of action, the forms in which it is applied, its concentration and the study trends in the world have been reviewed.

Chemistry of salicylic acid

SA is a group of plant phenols with an aromatic ring carrying a hydroxyl group or a functional derivative thereof and has been reported to play an important role in the regulation, growth and development of plant growth like other phenolic compounds (Özeker, 2005).

Salicylic acid (SA) as a signal molecule in plant systems

As a response to unfavourable conditions, signal molecules are synthesized and subsequently activate a range of signal transduction pathways in complex plant system. Furthermore, the plausible protective roles of the some signal molecules are known in helping plant to cope with the stress (Ganesan and Thomas, 2001). Calcium, jasmonic, ethylene and ethylene and salicylic acid are of the identified signalling molecules (El-Tayeb and Ahmed, 2010). Of the identified molecules, the protective roles of salicylic acid as a defence signal transducer or messenger under stress conditions has been well-reported (Raskin, 1992; Klessig and Malamy, 1994; Ganesan and Thomas, 2001). Greater economic yield of plants under water stress have been observed when plants were treated with SA (Gomez, 1993; Fayez and Bazaid, 2014; Kulak, 2016). Not limited to the response against abiotic stress factors, SA is considered as a likely signal disease resistance in plants (Raskin, 1992).

Why is the salicylic acid most preferred growth regulators? What is the advantage factor of salicylic acid over other growth regulators?

In recent years, many studies have intensively focused on SA due to SA's function as an endogenous signal mediator of local and systemic plant defence responses against biotic factors (Rivas-San Vicente and Plasencia, 2011). Also, for the exogenous chemicals applied to improve environmental induced perturbations, salicylic acid was found to be mostly preferred and used as a growth regulator. Of many signal molecules, SA is described as a hormone-like and phenolic-structured molecule and exhibiting vital role in the regulation of plant growth and development. This endogenous plant growth regulator is synthesized in low amounts. Note that SA should not be narrowly discussed as endogenous molecule but also defined, described and discussed as a potent molecule vital to plant systems once used an exogenous regulator under optimal conditions including optimal concentration, mode of applications, appropriate application time etc. for plants. Furthermore, the concentration in plant tissues is dynamic in response to any environmental stimuli. Its defence roles against biotic and abiotic stresses have been well-documented (Yalpani et al., 1993; Szalai et al., 2011). Regulation of physiological processes in plants such as stomatal closure, nutrient uptake, chlorophyll synthesis, protein synthesis, transpiration and photosynthesis has been partially attributed to the SA (Raskin, 1992).

What about the optimal concentration of salicylic acid? How important the concentration is? What about the application mode of salicylic acid? Foliar, priming or rooting media? At what intervals should salicylic acid be applied?

In the study by Kulak (2016), the role of SA priming against water stress in basil (*Ocimum basilicum* L.) was investigated. For determination of optimum SA-concentration for the subsequent water stress treatment, the basil seeds were soaked in different concentrations of SA including 0.001, 0.05, 0.10, 0.25, 0.50, 1.00, and 2.00 mM in dark at room conditions for 24 hours. Then the seeds were let germination for one week in Petri dishes filled with perlite. Salicylic acid concentrations that showed growth and development in the positive direction relative to the control group (0.00 mM) were used in water stress experiments at the next stage of study. SA concentrations to be used in the study were determined according to the germination percentage, seedling length and age weights of the seeds. According to the findings obtained for the determination of the optimum salicylic acid concentration, 0.05 and 0.1 mM salicylic acid concentration was obtained from the applications. The higher concentration caused adverse effects and consequently not used for the subsequent treatments (Table 1). The similar pre-treatment concentration determination by Kang et al. (2012), the concentration ranging from 0,1 to 3,00 mM was tested in wheat seedlings for 3 days. It was reported that 0.5 mM SA accelerated seedling growth and that other concentrations had adverse effects, particularly high concentrations (2.0 and 3.0 mM). The plant growth and development parameters are-in general-positively influenced with the application of the salicylic acid but at an optimal concentration. It is worthy to note that for each plant species, cultivars or genotypes, preliminary studies concerned with optimal concentration should be conducted and the toxic concentration must be removed for the subsequent processes of the work. Secondly, application time of the chemicals might also be taken into consideration.

Along with the studies listed in Table 2, the concentration range for exogenous applied SA against abiotic stress conditions is about (0.01-0.05 mM), (0.1-0.5 mM) and >1 mM. While pre-treatment with 0.01-0.05 mm exogenous SA is sufficient for significant increase of cold tolerance for wheat and tomato (Ding et al., 2002), the optimal concentration level for increasing stress tolerance usually ranges from 0.1 mm to 0.5 mm for most low-level-SA plants (reviewed by Yuan and Lin, 2008). However, it may not be correct to generalize the results from studies because of the fact that the experimental parameters such as plant species, developmental stages of the investigated plant, duration of the experiment, mode of SA-application, frequency of applications, stress conditions (stress type, severity, frequency, duration or combination of more stress) should be considered, not ignored.

Table 1. Changes in basil seeds primed with salicylic acid*

	Germination (%)		Length (cm)		Fresh Weight (mg)	
SA (mM)	Mean	Change (%)	Mean	Change (%)	Mean	Change (%)
0	89.98b	100	5.07c	100	98.68b	100
0.05	100.00a	111.14	7.0 a	138.66	115.68a	117.23
0.1	94.44ab	104.96	6.13b	120.91	91bc	92.22
0.25	67.78c	75.33	4.00d	78.9	80.68cd	81.76
0.5	58.89d	65.45	3.6d	71.01	76.33d	77.35
1	52.22de	58.04	2.17e	42.8	62.67e	63.51
2	45.55e	50.62	1.97e	38.86	58.00e	58.78

*The results were retrieved from the study by Kulak (2016); Values were mean. Data with different letters in the same column indicate a significant difference at $P < 0.05$ level.

How are the protective mechanisms of salicylic acid?

It has been well-documented that plants exhibit different defence mechanisms including enzymatic and non-enzymatic antioxidant systems in order to cope with stressors or sustain their normal life span. Plants themselves like other livings are perpetual factories and their health and sustainability are dependent on the anabolic and catabolic processes. The proper or improper functions of those processes are consequences of antioxidant systems coupled with antioxidant enzymes and through substances other than antioxidant enzymes. SA-induced suppression of antioxidant enzyme system in plants exposed to Cd-toxicity has been reported. However the protective roles of SA were also highlighted. The protective activity in response to the Cd-toxicity was considered as through substances other than antioxidant enzymes (Metwally et al., 2003). For the sequestration or detoxification of Cd in plants, synthesis of proteins or differing abundance of primary and secondary metabolites may be regarded as protective or inhibitory mechanisms of plants. In order to find unhidden answer for plant systems, many approaches, especially preliminary studies, are milestones directing the way of experiments or pioneering the experimental parameters or tests for the answers through new perspectives in order not to report the repeated results.

One more important issue is about the transportability of SA in plant systems. The questions “In which parts of the plant is salicylic acid produced? When and where is salicylic acid transferred when the plant is exposed to the stress factors?” are great interest for the researchers. In excellent review by Raskin (1992), it has been reported that no clear direct evidence to be used for proving the transportability of SA because of the fact that SA could be transported, metabolized and/or conjugated in complex plant systems due to its physical properties. Furthermore, what happens to the exogenously applied SA and endogenous content of SA? The exogenously applied SA is carried away from its initial application to the other plant tissues for plant response generation.

Table 2. Some studies concerned with the salicylic acid applications

Study	Plant species	Developmental stage	Duration of experiment	Mode of application	Frequency	Conc. (mM)	Experiment
Ananieva et al. (2002)	Barley	12-days-old seedlings	1,2,3, and 6 hours	Rooting	Once	0.5	Paraquat tolerance
Kang et al. (2003)	Banana	11-cm high seedling	3-days treatment	Pre-spray and rooting	Once	0.5	Chilling tolerance
Waseem et al. (2006)	Wheat	Germinated seeds	45-days treatment	Rooting	Once	0.036; 0.072	Drought tolerance
Taşgın et al. (2006)	Wheat	7-days-old seedling	38-days treatment	Spray	Once	0.01	Cold tolerance
Jing et al. (2007)	Rice	Seventh leaf developed	20-days treatment	Rooting	Once	0.1	Lead stress tolerance
Popova et al. (2009)	Pea	Seed	12-days treatment	Priming	6 hours	0.5	Cd- toxicity
El Tayeb and Ahmed (2010)	Wheat	Seed	2- weeks treatment	Priming	6 hours	0.5	Drought tolerance
Szepesi et al. (2011)	Tomato	Seedling	3-weeks treatment	Rooting	Once	0.1	Hardening processes
Nazar et al. (2011)	Mungbean	15-days-old seedling	15-days treatment	Spray	Once	0.1; 0.5;1.0	Salinity tolerance
Dong et al. (2011)	Cucumber	18-days-old seedlings	20-days treatment	Rooting	3, 6, 9, 12 days	0.01; 0.05;0.1	Salinity tolerance
Mirabi and Hasanabadi (2012)	Tomato	Seed	14-days treatment	Priming	48 hours	0.43	Priming effect
Kang et al. (2012)	Wheat	2-weeks-old seedlings	3-days treatment	Rooting	Once	0.5	Salinity tolerance
Sharafizad et al. (2013)	Wheat	seed	7-days treatment	Priming	24 hours	0.7;1.2; 2.7	Drought tolerance
Saidi et al. (2013)	Bean	4-days-old seedling	10-days treatment	Rooting	Once	0.01; 0.05; 0.1	Cd-toxicity
Orabi et al. (2013)	Faba bean	45-days and 60 day-old seedling	120-days treatment	Spray	Twice (45 and 60 days)	1.0; 2.0	Salinity tolerance
Guo et al. (2013)	Bluegrass	Seed	25-days treatment	Priming	12 hours	0.5	Cd-toxicity
Fayez and Bazaid (2014)	Barley	3-weeks-old seedling	2- weeks treatment	Spray	Once	0.05	Drought and salinity tolerance
Agami (2013)	Maize	Seed	60-days treatment	Priming	12 hours	0.1	Salinity tolerance

Does endogenous SA content vary with exogenous applied SA or change with biotic and abiotic stress factors?

The endogenous regulatory roles of SA as an important plant hormone regarding with local and systemic disease resistance and an induced of pathogenesis-related (PR) proteins have been hypothesized and proven using many approaches. The induced increase in resistance against virus and PR-1 proteins were positively correlated with the tobacco tissue levels of SA (Yalpani et al., 1993). Moreover, endogenous SA content was increased in whitefly-infested plants upon Agrobacterium inoculation. Augmented SA content involved in whitefly-derived plant defence against *Agrobacterium* (Song et al., 2015).

Salicylic acid-accumulating mutants of *Arabidopsis* (acd6 and cpr5) were more tolerant to drought stress than wild-type plants and drought tolerance related genes were more expressed in mutant plants. The results suggest that accumulation of endogenous SA confers drought tolerance to *Arabidopsis* (Okuma et al., 2014). Chilling tolerance in cucumber seedlings was also correlated with endogenous SA content (Dong et al., 2014).

Involvement of endogenous salicylic acid in iron-deficiency responses in Arabidopsis was reported. Accumulation of SA content coupled with Fe deficiency elevated auxin and ethylene signalling, thereby activating Fe translocation through transcriptional regulation of downstream Fe genes (Shen et al., 2016).

Following exogenous SA treatment, free SA content decreased at most sampling points after application of SA treatment in *Scutellaria baicalensis* (Hu et al., 2017). Endogenous SA level in pre-treated pea seeds increased but the augmented endogenous SA levels in plant tissues did not originate from the exogenous SA. Measured SA content was reported to be product of de novo synthesis, rather than taken up and mobilized by the plants (Szalai et al., 2011). SA pre-treatment improved and controlled the ability of plants to regulate the endogenous levels of SA when exposed salt stress but under suitable nutrient conditions (Kim et al., 2017).

Why bibliometric analysis?

As a methodology, the VOSviewer tool was used to visualize the results. The documents including salicylic acid keyword but limited to agricultural and biological sciences were extracted from Scopus database. Briefly, research was done using salicylic acid keyword in article title and then 46,454 documents were retrieved on December 20, 2017. The second step was limitation of the results to agricultural and biological sciences. In this context, 1422 documents relevant and available peer-reviewed publications were analysed. According to the systematic analysis, 4688 authors, 2880 keywords and 84 countries were determined. After inclusion of some criteria, those numbers were reduced. The annual trends of publications in the field of research, considering the number of documents, number of authors, levels of collaborations among authors and countries, year publications and core publishing journals were analysed.

According to the analysis results, there was an increase trend in number of publications concerned with salicylic acid in agriculture and biological sciences. The highest number of documents was observed in 2017 with a total of 701 publications. The first publication in the investigated field of study was in 1887 published in Transactions of the American Fisheries Society by A. Howard Clark. The study was about fish preservation by the use of acetic, boracic, salicylic, and other acids and compounds.

Plant Physiology, Plant Journal, Plos One, Molecular Plant Microbe Interactions, Frontiers in Plant Science, Plant Cell, Journal of Experimental Botany, Plant Physiology and Biochemistry, Journal of Plant Physiology and Planta are of the core publishing journals.

What do the terms extracted from documents tell us?

According to the systematic analysis, 4688 authors, 2880 keywords and 84 countries were determined. After inclusion of some criteria, those numbers were reduced. Along with the results, two main salicylic acid research clusters according to the most relevant terms were identified. First cluster was composed of abiotic stress terms and related antioxidant activity and enzymes. The first cluster can be considered as biochemistry and abiotic stress. The second cluster was composed of biotic stress factors and related plant immunity terms and molecular approaches. So, the second cluster can be considered as molecular biology and biotic stress (Figure 1).

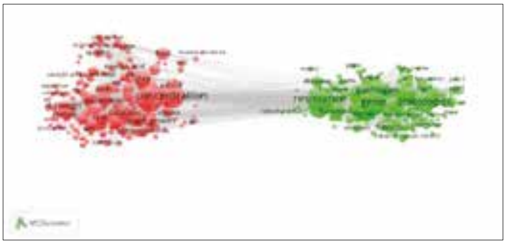

Figure 1. The most relevant and co-occurrence of terms retrieved from the studies

What do the terms proposed by authors in the documents analysed tell us? Can they be pioneer for the future studies?

The simple keyword extraction provides raw information about the research topics but they are assigned to documents to represent the core content of their papers. In this regard, keyword analysis can be used to determine the progress the research frontiers associated with a knowledge (He, 1999), proposing the gap of keyword analysis in SA uses in researches in agricultural and biological studies. Herewith the core results, this section should be considered as the most important contribution of the manuscript. Co-occurrence and author keywords might be considered as one of the factors to provide information on SA and its most uses in many respects in agricultural and biological sciences. In the current study, bibliometric analysis presented nine clusters concerned with keywords proposed by the authors. Of the clusters, economically and nutritionally significant plants (wheat, barley, sunflower, maize, soybean etc.) have been investigated for their tolerance against abiotic stress conditions combined with salicylic acid applications. In this context, proline content, malondialdehyde content and antioxidant enzymes like parameters have been used for assessment of tolerance. In many studies, basic physiological and biochemical assays have been performed. However, of the main clusters, tobacco plant have been extensively used for biotic stress-induced with virus. Along with those studies, local and systemic disease resistance and an induced of pathogenesis-related (PR) proteins have been hypothesized and proven using many approaches. Regulation of PR proteins has been correlated with salicylic acid contents. In those biotic stress studies, molecular approaches, especially gene expression levels, have been highlighted (Figure 2). To conclude, results obtained from abiotic stress researchers are needed and proven using transcriptomic and proteomic approaches.

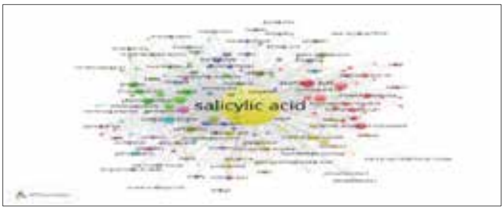

Figure 2. The keywords proposed by the researchers

Top productive countries and authors

China and United States are of the first two-most productive countries. The studies disseminated by researchers from United States have been mostly referenced and total link strength of United States also highest, as well (Figure 3, Table 3). Of the authors, Klessig D.F. (53), Baldwin I.T. (32), Métraux J.P. (31), Pieterse C.M.J. (29), Shah J. (29), Ohashi Y. (28), Seo S. (24), Hwang B.K. (23), Janda T. (23) and Chen Z. (21) are of the most productive authors.

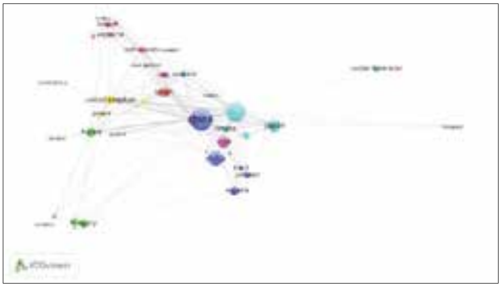

Figure 3. Top productive countries

Table 3. Top ten publishing countries

	Country	Documents	% of Total Documents	Citations	Citation Per Document	Total Link Strength
1	China	303	21.31	5487	18.11	57
2	United States	229	16.10	17485	76.35	144
3	India	144	10.13	2623	18.22	23
4	Japan	105	7.38	4550	43.33	50
5	Iran	93	6.54	794	8.54	11
6	Germany	78	5.49	4481	57.45	71
7	Spain	70	4.92	2532	36.17	35
8	United Kingdom	64	4.50	4733	73.95	64
9	France	52	3.66	2360	45.38	40
10	Egypt	50	3.52	729	14.58	17

Future outlook

In this section, it was focused on the gaps and was given suggestions for future studies associated salicylic acid studies, especially on the abiotic stress and salicylic acid interactions.

1. Biochemical and physiological aspects of SA-triggered increase in tolerance versus abiotic stress factors have been well documented and the studies. The most of the studies are based on the plant species rich for their nutritional and economic values. There are lacking studies concerned with molecular basis of salicylic acid combined abiotic stress conditions. In this context, mechanism-enlightening studies coupled with omic approaches are required.

2. An improvement regarding with increase in tolerance of plants versus stress conditions using salicylic acid have been extensively reported but the studies about secondary metabolite- not only reporting total content of secondary metabolites but also profiling the metabolites essential-are required. Sub-sequential studies may be concentrated on their molecular basis and further desired crop

improvement with high tolerant and quality content.
3. The most favourable conditions including application time, mode of application, application doses or duration of priming should be optimized for each plant species.

Highlights of the study

Along with the present study, it was illustrated a schema as studies regarding to: **i)** the current state knowledge of salicylic acid studies of profiling the key areas of the salicylic acid uses; **ii)** pointing out the stages of development of the studies; **iii)** presenting assessments for the significance of the studies performed; **iv)** giving the results through vote-counting method; **v**) giving suggestions for the key areas for further work.

Limitations of the study

Although this is the first study-up to best survey-to present the salicylic acid containing studies in field of agricultural and biological sciences from the largest existing database using VOSviewer program, we have several limitations in this study. **i**) The data was only extracted from SCOPUS and so documents in non-indexed plant journals have not been considered. **ii**) The search was then restricted for publications that contain the words "salicylic acid" in the title and abstracts as well as the search was then restricted to the agricultural and biological sciences. **iii**) Hence some publications might not contain salicylic acid and related terms in the publications title and abstracts, so it is possible that not all publications for salicylic acid including studies were identified. It is worthy to consider and state that there are many studies deciphering and describing the structure and roles of salicylic acid. The study can be considered as vote-counting review paper.

CONCLUSIONS

The main conclusions of the review have been listed as below:
1. There was an increase trend in number of publications concerned with salicylic acid in agriculture and biological sciences. The highest number of documents was observed in 2017 with a total of 701 publications.
2. China and United States are of the first two-most productive countries.
3. Two main salicylic acid research clusters according to the most relevant terms were identified. First cluster was composed of abiotic stress terms and related antioxidant activity and enzymes. The second cluster was composed of biotic stress factors and related plant immunity terms and molecular approaches.
4. While crop plants such as wheat, barley, sunflower, maize and soybean have been investigated for their tolerance against abiotic stress conditions, tobacco has been extensively used for biotic stress related studies.

Finally, it can be stated that the studies on salicylic acid uses in agricultural and biological studies have been directed in two different ways for stress conditions. Interestingly, abiotic stress studies are mostly limited to the basic physiological and biochemical assays. The similar results have been reported in most of publications. On the other hand, biotic stress researches are mostly concentrated on virus-tobacco interactions and subsequent analysis of transcription levels of pathogenesis-related proteins.

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